KaryoStudio v1.2
User Guide

Software for Cytogenetics Data Analysis and Reporting

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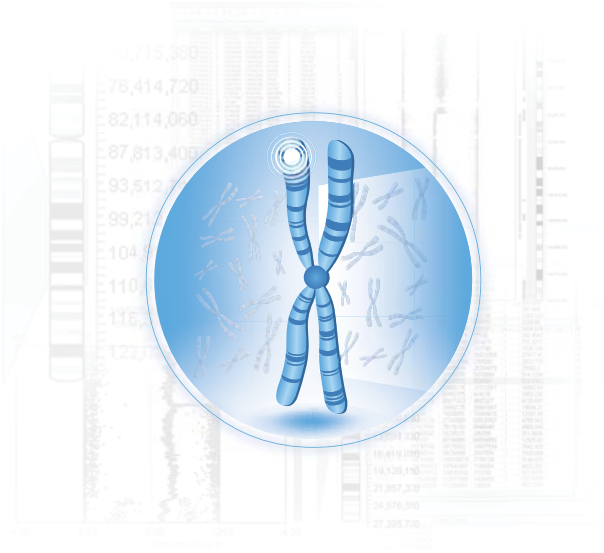

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Chapter 1
Overview

Topics

2 Introduction
2 Audience and Purpose
3 Installing KaryoStudio
6 KaryoStudio Workflow

Introduction

Cytogenetics is an area of genetics that focuses on chromosomal aberrations resulting in various conditions and phenotypes. It includes the routine analysis of G-banded chromosomes (karyotyping), as well as molecular cytogenetics such as fluorescent *in situ* hybridization (FISH) and comparative genomic hybridization (CGH).

In recent years, many forms of cytogenetics have moved to array-based technologies. Because of the added benefit that SNP information can provide, the field has begun to incorporate such information into their research.

This user guide describes Illumina's KaryoStudio cytogenetics software. KaryoStudio allows researchers to analyze data generated from Illumina's Infinium® DNA Analysis BeadChips and generate *.pdf reports from this data.

KaryoStudio software is for research use only.

Audience and Purpose

This guide is written for researchers who want to use Illumina's KaryoStudio software to analyze data generated from Illumina's Infinium assays, to identify cytogenetic or structural aberrations, and to generate *.pdf reports from this data.

KaryoStudio has been designed specifically for cytogeneticists who are using Illumina’s Infinium products to detect aberrations in the genome. KaryoStudio accepts Infinium data, automatically performs normalization, scans data for aberrations, displays the analyzed data allowing interpretation by the use, and generates reports useful for interpreting results. The software is geared towards finding aberrations that are 75kb and larger, and cross-matching those aberrations with information from public databases.

KaryoStudio is standalone software application, separate from Illumina's GenomeStudio® software suite. If you would like to obtain comprehensive genotyping information from data generated using Infinium products, or perform a more customized data analysis, you may want to use the GenomeStudio Genotyping Module. For more information about this and other Illumina software products, contact your local account manager or visit www.illumina.com.

Installing KaryoStudio

If you do not have KaryoStudio v1.0 installed on your computer, you can immediately install KaryoStudio v1.2 from the CD.

If you are a KaryoStudio v1.0 user, you must uninstall this version from your computer before you can install KaryoStudio v1.2. If you do not uninstall KaryoStudio v1.0 before attempting to uninstall KaryoStudio v1.2, you will be prompted to do so.

You may also need to uninstall the cnvPartition algorithm you are currently using. cnvPartition v2.4.4 will be installed automatically with KaryoStudio v1.2.

To install KaryoStudio, perform the following steps:

1. Put the KaryoStudio CD into your CD drive.

   If the Illumina KaryoStudio Installation screen appears, continue to Step 2.

   If the CD does not load automatically, double-click the KaryoStudio<version>.exe icon in the KaryoStudio folder on the CD.

The CD includes two versions of KaryoStudio.
If you are installing KaryoStudio on a 32-bit computer, click *KaryoStudio.exe*.
If you are installing KaryoStudio on a 64-bit computer, click *KaryoStudio64.exe*.

The KaryoStudio application unzips and the KaryoStudio application icon

appears on your desktop.

2. Double-click the KaryoStudio icon on your desktop. If this is a new installation, the Registration screen appears (Figure 1).

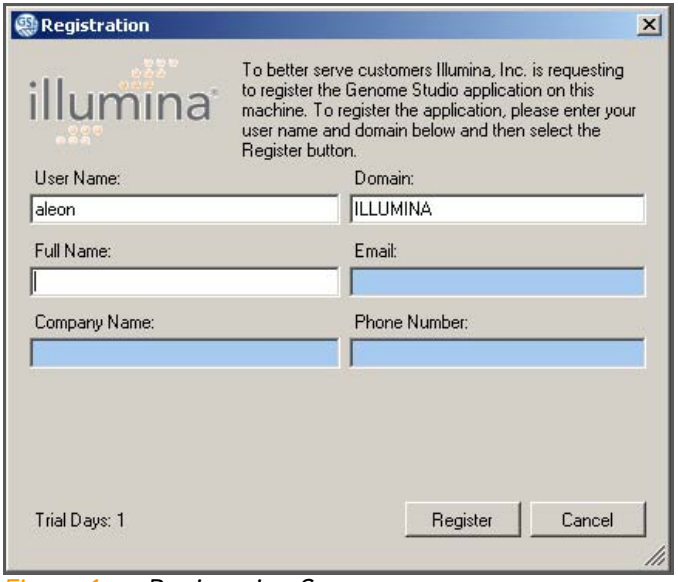

Figure 1 Registration Screen

If you have previously installed and registered another version of KaryoStudio, the registration screen does not appear.

The Registration Screen has a GenomeStudio (GS) icon and refers to Illumina's GenomeStudio software suite. This is because KaryoStudio, while separate from GenomeStudio, uses the same registration database.

3. Complete the following information:
   - **User Name**--Enter the user name you use to sign into your computer

- **Domain**--KaryoStudio recognizes your network domain and populates this field automatically.
- **Full Name**--Enter your full name.
- **Email**--Enter your email address.
- **Company Name**--Enter the name of your organization (company, institute, university, etc.).
- **Phone Number**--Enter your phone number.

NOTE

Illumina uses this information for Technical Support purposes.

4. Click **OK**.

   KaryoStudio is registered (Figure 2), and you can continue the installation process.

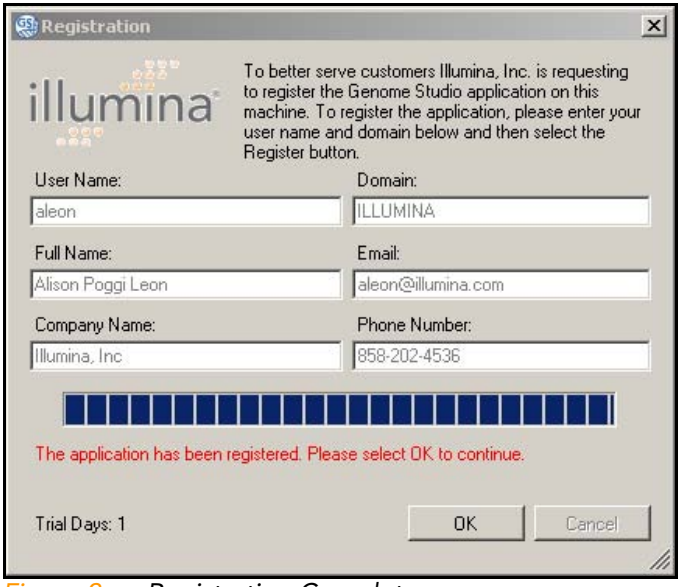

Figure 2 Registration Complete

5. Click **OK**.
6. KaryoStudio is installed on your computer and opens to the main window (Figure 3).

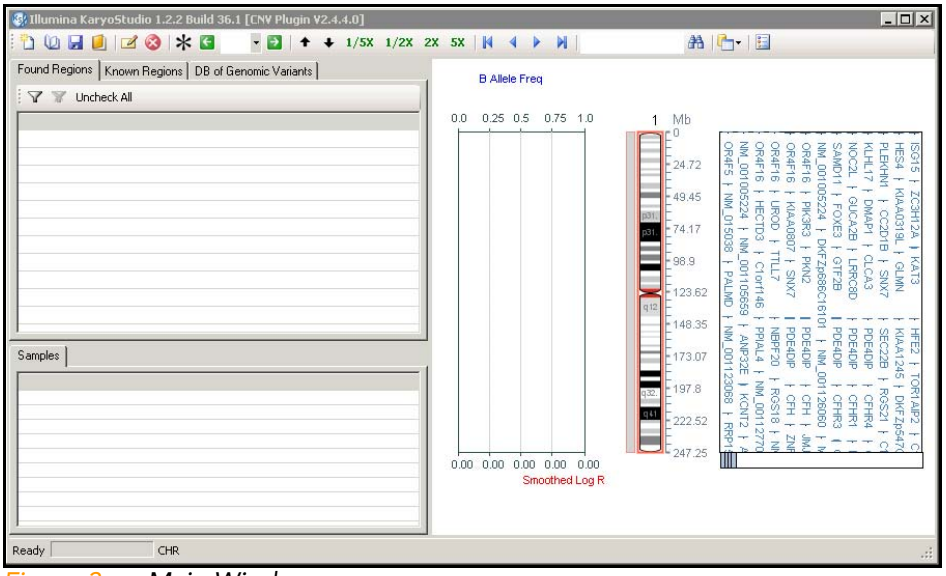

Figure 3 Main Window

From this screen, you can create a new KaryoStudio project, open an existing project, view your data, generate reports, and more. Continue to Chapter 2 for information about how to create a KaryoStudio project.

KaryoStudio Workflow

A typical data visualization and analysis workflow using KaryoStudio includes the following steps:

1. Use the project wizard to load data into KaryoStudio.
2. Scan the data for aberrations using KaryoStudio.
3. Adjust the filter settings to display found regions of interest.
4. Display found regions of interest in the chromosome browser and Found Regions table.
5. Cross-match found regions to areas of the genome known to be associated with various conditions or phenotypes.
6. Select report settings.
7. Generate a KaryoStudio Report.

Chapter 2
Loading Your Data

Topics

8 Introduction

8 Creating a New Project

14 Loading Sample Intensity Data Using a Sample Sheet

21 Loading Sample Intensity Data by Selecting Directories

30 Opening an Existing Project

30 Assessing the Quality of Your Data

Introduction

Using the Project Wizard is an easy way to begin a new KaryoStudio project.

Before you create a project, make sure that you have the following files easily accessible in a known location on your computer:

- Intensity data files (*.idat files) from a BeadChip (or BeadChips) of interest
- A product manifest (*.bpm file) specific to the BeadChip product used
- A product cluster file (*.egt file) specific to the BeadChip product used

Creating a New Project

To create a new KaryoStudio project, perform the following steps:

1. On the KaryoStudio main screen, click **New Project** to open the Project Wizard.

The Welcome screen appears (Figure 4).

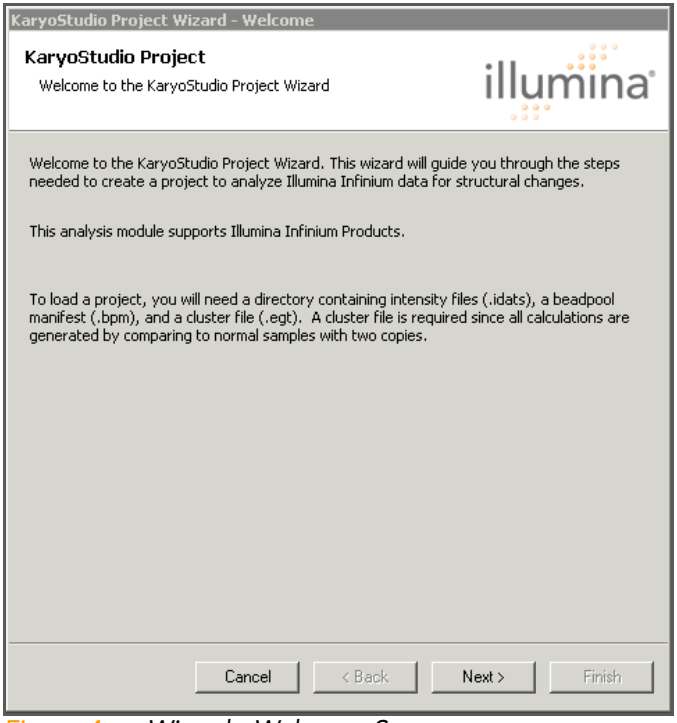
KaryoStudio Project Wizard - Welcome
KaryoStudio Project
Welcome to the KaryoStudio Project Wizard
illumina
Welcome to the KaryoStudio Project Wizard. This wizard will guide you through the steps needed to create a project to analyze Illumina Infinium data for structural changes.
This analysis module supports Illumina Infinium Products.
To load a project, you will need a directory containing intensity files (.idats), a beadpool manifest (.bpm), and a cluster file (.egt). A cluster file is required since all calculations are generated by comparing to normal samples with two copies.
Cancel
< Back
Next >
Finish

Figure 4 Wizard - Welcome Screen

2. Click **Next**.

The Project Location screen appears (Figure 5).

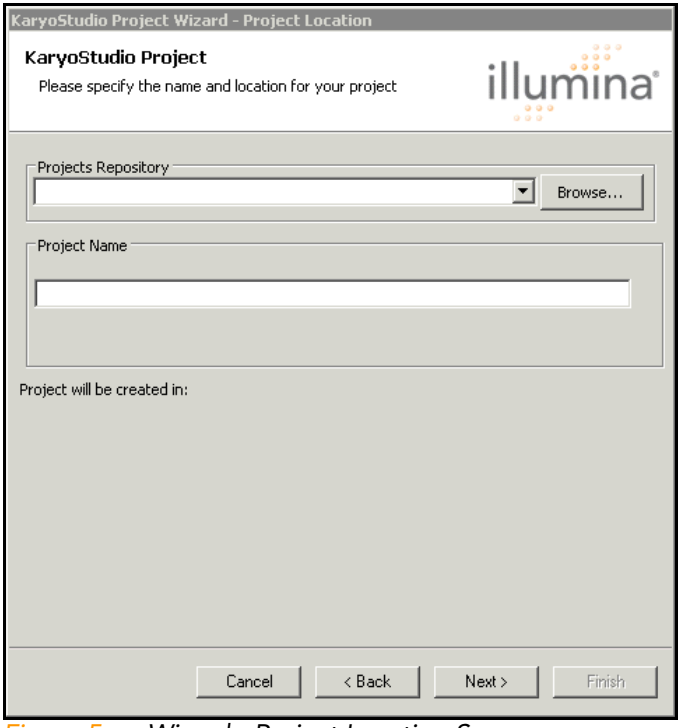

Figure 5 Wizard - Project Location Screen

3. To select the repository folder where you would like to store your project, do one of the following:
   - Enter a location in the Project Repository field
   - Browse to a location (Figure 6).

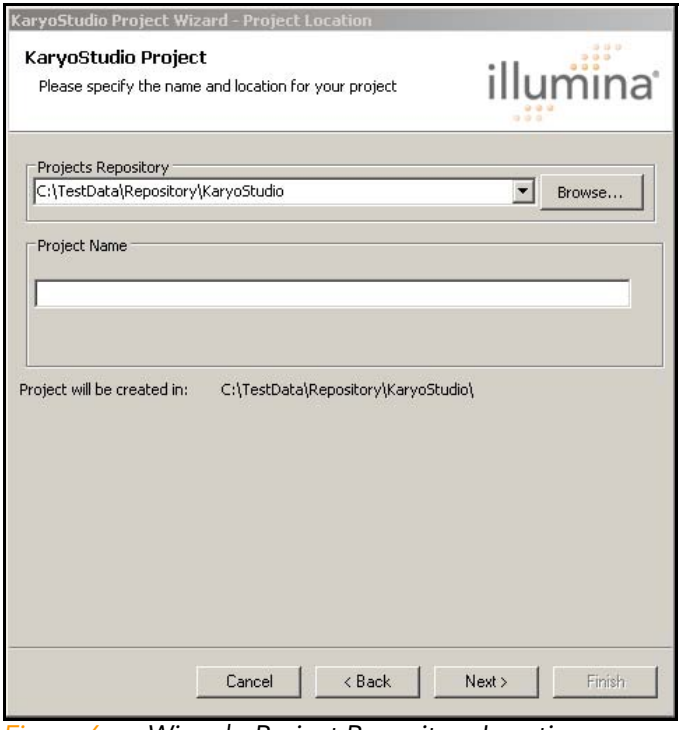

Figure 6 Wizard - Project Repository Location

4. In the Project Name area, enter a name for your project in the text field (Figure 7).

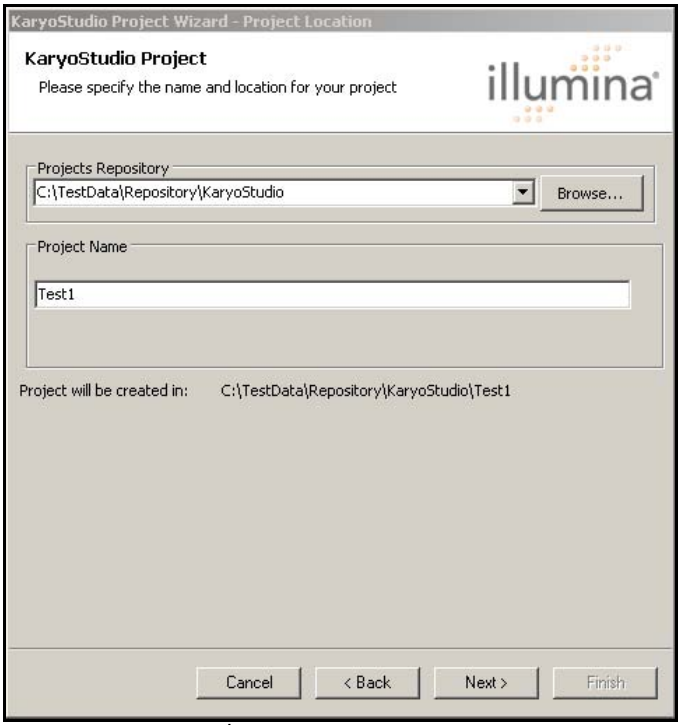

Figure 7 Wizard - Project Name

5. Click **Next** to advance to the Loading Sample Intensities screen (Figure 8).

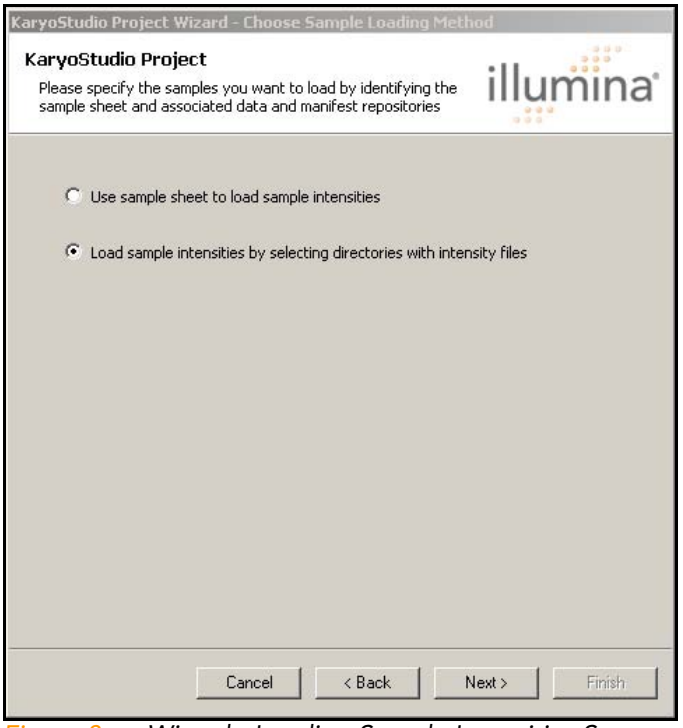
KaryoStudio Project Wizard - Choose Sample Loading Method
KaryoStudio Project
Please specify the samples you want to load by identifying the sample sheet and associated data and manifest repositories
illumina
Use sample sheet to load sample intensities
Load sample intensities by selecting directories with intensity files
Cancel
< Back
Next >
Finish

Figure 8 Wizard - Loading Sample Intensities Screen

6. Select one of the following options:
   - Select **Use sample sheet to load intensity data** if you have a sample sheet that you want to use with this project.

     After selecting this method, continue to *Loading Sample Intensity Data Using a Sample Sheet* on page 14 to finish creating the project.
   - Select **Load sample intensities by selecting directories with intensity data** if you want to load project data directly from intensity data files.

     After selecting this method, continue to *Loading Sample Intensity Data by Selecting Directories* on page 21 to finish creating the project.

NOTE

You must use a sample sheet for optimal analysis of sex chromosomes. KaryoStudio requires the gender information imported in the sample sheet in order to correctly call found regions or normal regions on the X and Y chromosomes. Without a sample sheet, data plots will be accurate, but found regions may not be.

Loading Sample Intensity Data Using a Sample Sheet

Perform the following steps to load sample intensity data using a sample sheet.

1. From the Choose Sample Loading Method screen, click **Next** to advance to the Loading Sample Intensities Screen (Figure 9).

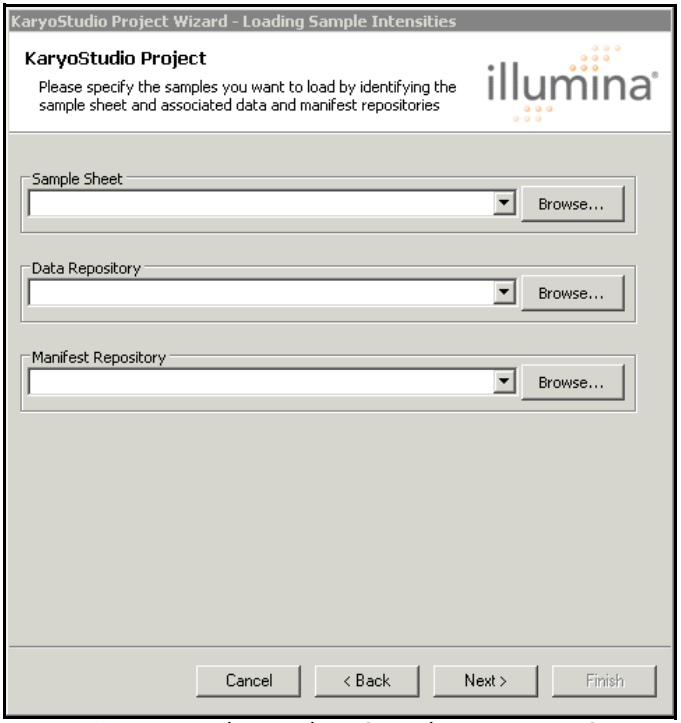

Figure 9 Wizard - Loading Sample Intensities Screen (Sample Sheet)

2. Browse to a directory that contains the sample sheet you want to use with this project (Figure 18).

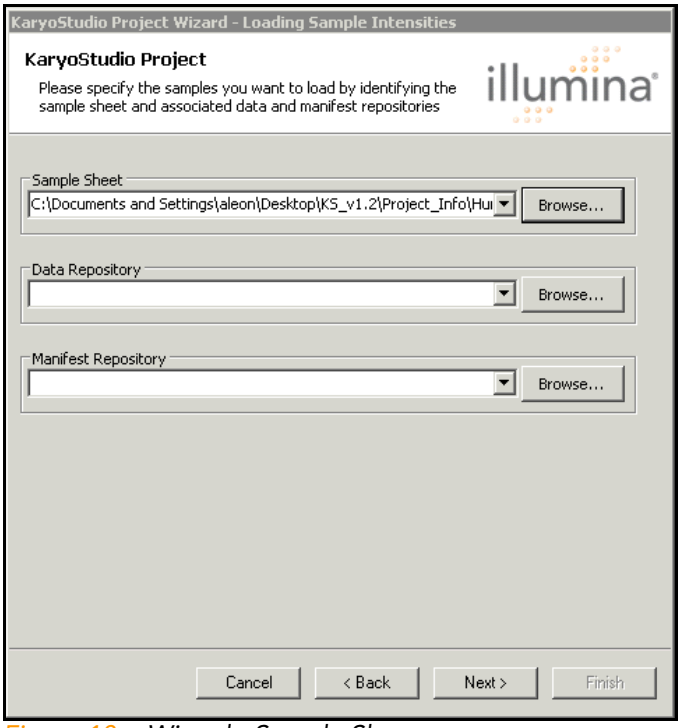

Figure 10 Wizard - Sample Sheet

3. Browse to the repository that contains the data you want to include in this project (Figure 19).

NOTE

You must specify a path in the wizard. However, if a different path is specified in the sample sheet, the path in the sample sheet overrides the path you provide in the wizard.

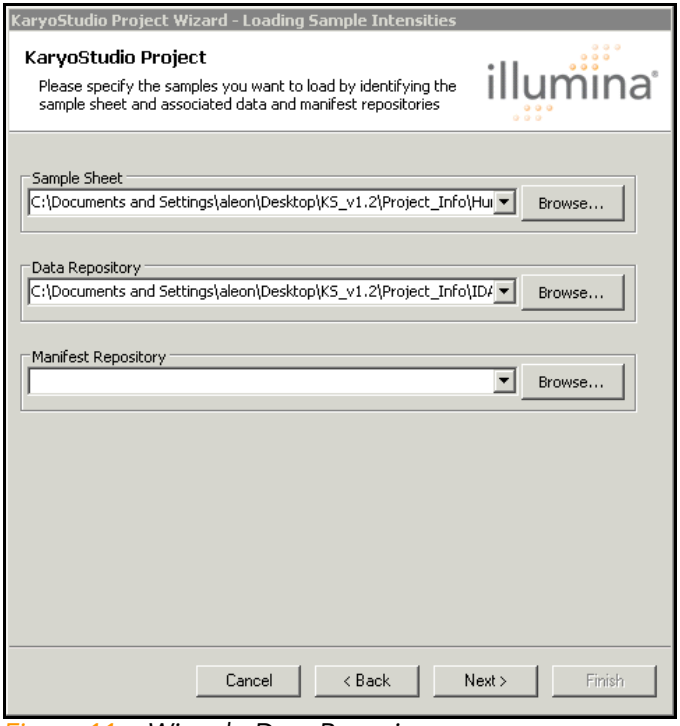

Figure 11 Wizard - Data Repository

4. Browse to the repository that contains the SNP manifest you want to use with this project (Figure 12).

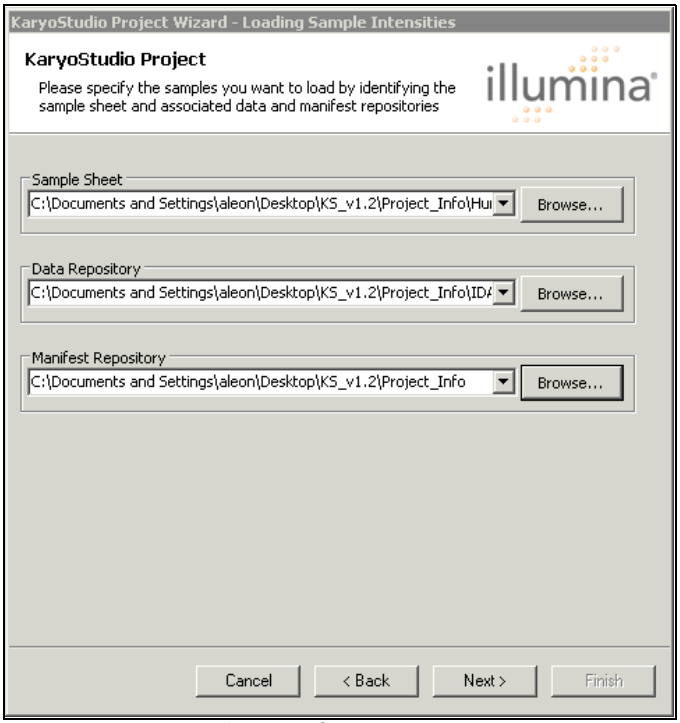

Figure 12 Wizard - Manifest Repository

5. Click **Next** to advance to the Cluster Positions screen (Figure 13).

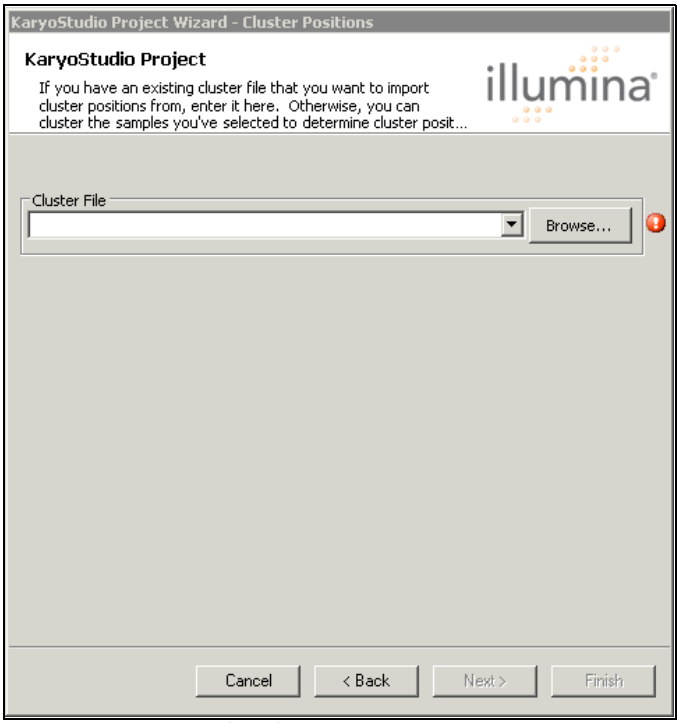
KaryoStudio Project Wizard - Cluster Positions
KaryoStudio Project
If you have an existing cluster file that you want to import cluster positions from, enter it here. Otherwise, you can cluster the samples you've selected to determine cluster posit...
illumina
Cluster File
Browse...
Cancel
< Back
Next >
Finish

Figure 13 Wizard - Cluster Positions

6. Browse to the cluster file you want to use with this project (Figure 14).

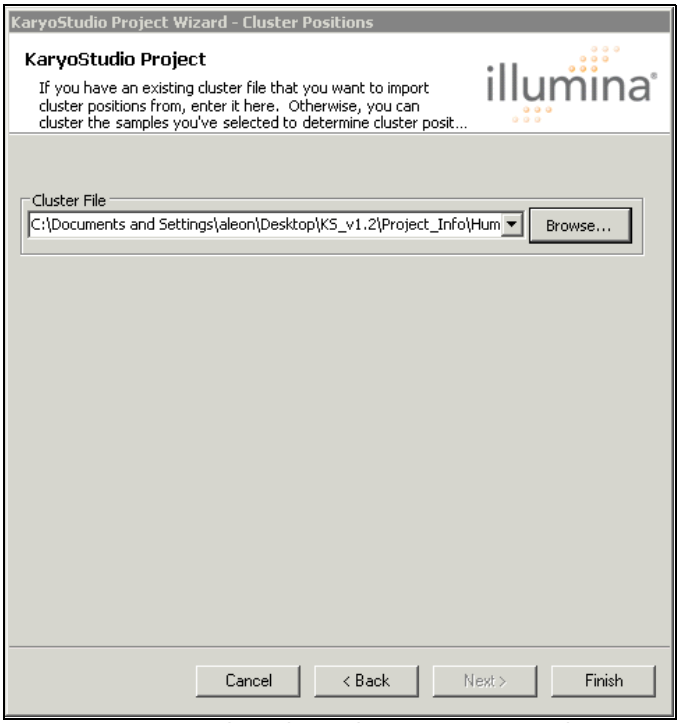
KaryoStudio Project Wizard - Cluster Positions
KaryoStudio Project
If you have an existing cluster file that you want to import cluster positions from, enter it here. Otherwise, you can cluster the samples you've selected to determine cluster posit...
illumina
Cluster File
C:\Documents and Settings\aleon\Desktop\KS_v1.2\Project_Info\Hum
Browse...
Cancel
< Back
Next >
Finish

Figure 14 Wizard - Select Cluster Positions File

7. Click **Finish**.

   KaryoStudio displays a progress bar while it loads the intensity files, manifest file, and cluster files, and runs the cnvPartition algorithm on the data you chose to include in this project (Figure 23).

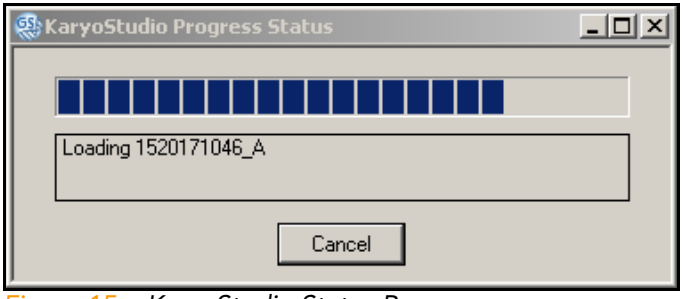

Figure 15 KaryoStudio Status Bar

NOTE

The amount of time it takes to create your project is directly related to the Infinium product you are using and the number of samples you are processing.

For example, it takes longer to load and analyze data from an Infinium HumanOmni1-Quad BeadChip than it does to load and analyze data from a HumanCytoSNP-12 BeadChip.

For more information about processing time, see the KaryoStudio System Information and Benchmark Performance Technical Note.

Once the data are finished processing and loading, KaryoStudio displays the new project (Figure 24).

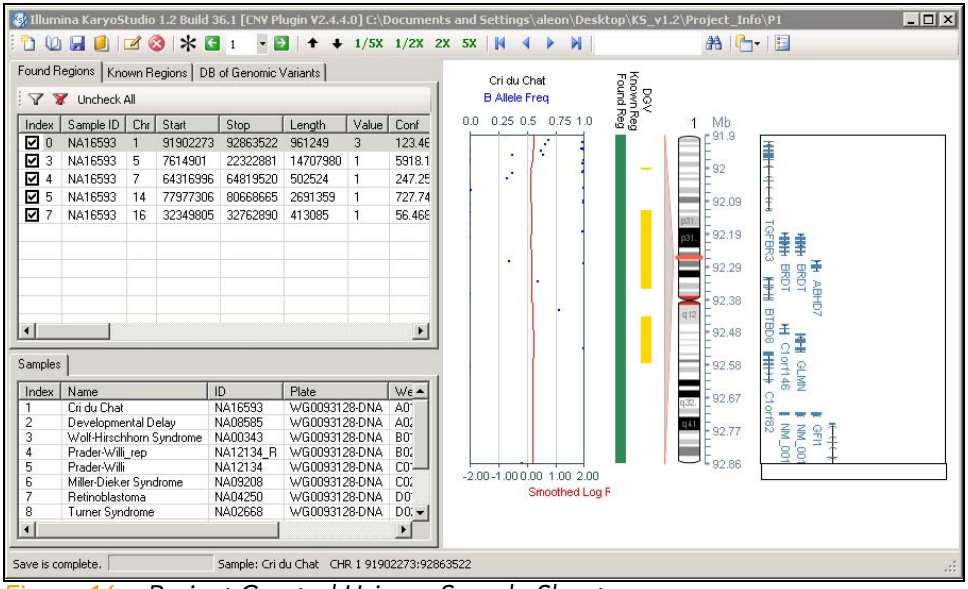
Illumina KaryoStudio 1.2 Build 36.1 [CNV Plugin V2.4.4.0] C:\Documents and Settings\aleon\Desktop\KS_v1.2\Project_Info\P1
Found Regions
Known Regions
DB of Genomic Variants
Uncheck All
Index Sample ID Chr Start Stop Length Value Conf
0 NA16593 1 91902273 92863522 961249 3 123.4E
3 NA16593 5 7614901 22322881 14707980 1 5918.1
4 NA16593 7 64316996 64819520 502524 1 247.25
5 NA16593 14 77977306 80688665 2691359 1 727.74
7 NA16593 16 32349805 32762890 413085 1 56.46E
Samples
Index Name ID Plate
1 Cri du Chat NA16593 WG0093128-DNA
2 Developmental Delay NA08585 WG0093128-DNA
3 Wolf-Hirschhorn Syndrome NA00343 WG0093128-DNA
4 Prader-Willi_rep NA12134_R WG0093128-DNA
5 Prader-Willi NA12134 WG0093128-DNA
6 Miller-Dieker Syndrome NA09208 WG0093128-DNA
7 Retinoblastoma NA04250 WG0093128-DNA
8 Turner Syndrome NA02668 WG0093128-DNA
Cri du Chat
B Allele Freq
Known Reg
Found Reg
DGV
Mb
Smoothed Log R
Save is complete.
Sample: Cri du Chat CHR 1 91902273:92863522

Figure 16 Project Created Using a Sample Sheet

Continue to *Assessing the Quality of Your Data* on page 30 for additional information.

Loading Sample Intensity Data by Selecting Directories

Perform the following steps if, instead of using a sample sheet, you prefer to load sample intensity data by selecting the directories that contain the data.

NOTE

If you want to include gender data for the samples in a KaryoStudio project, you must create the project using a sample sheet that includes gender data for the samples.

1. From the Choose Sample Loading Method screen, click **Next** to advance to the Loading Sample Intensities screen (Figure 17).

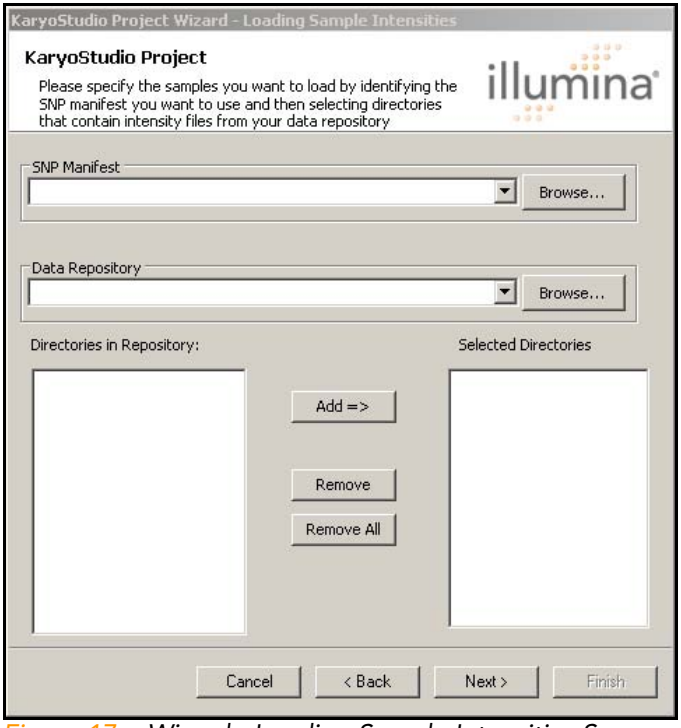

Figure 17 Wizard - Loading Sample Intensities Screen (Directories)

2. Browse to a directory that contains the SNP manifest you want to use with this project (Figure 18).

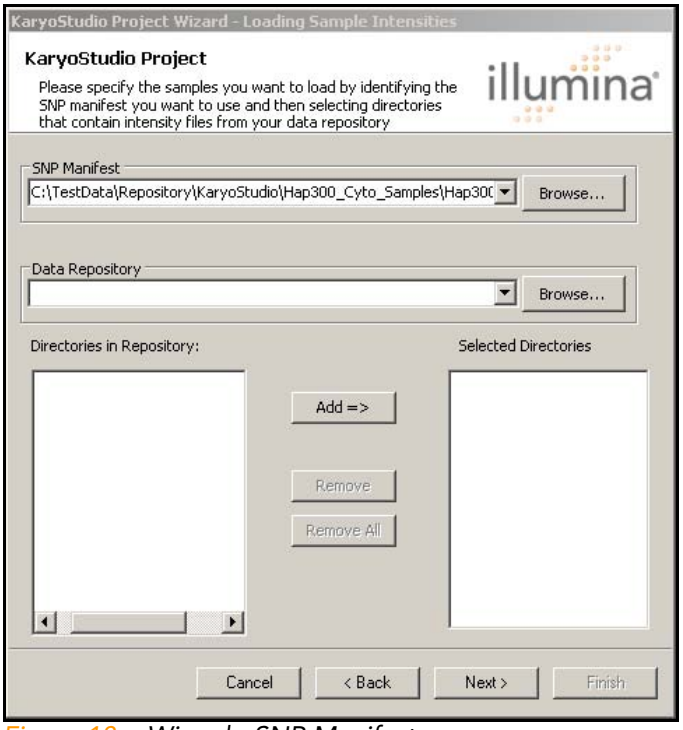

Figure 18 Wizard - SNP Manifest

3. Browse to the data repository that contains the data you want to include in this project.

   The directories available for selection appear in the Directories in Repository area (Figure 19).

NOTE

KaryoStudio accepts manifests only from a single Infinium product type. You cannot create a KaryoStudio project with multiple products, or with different versions of the same product.

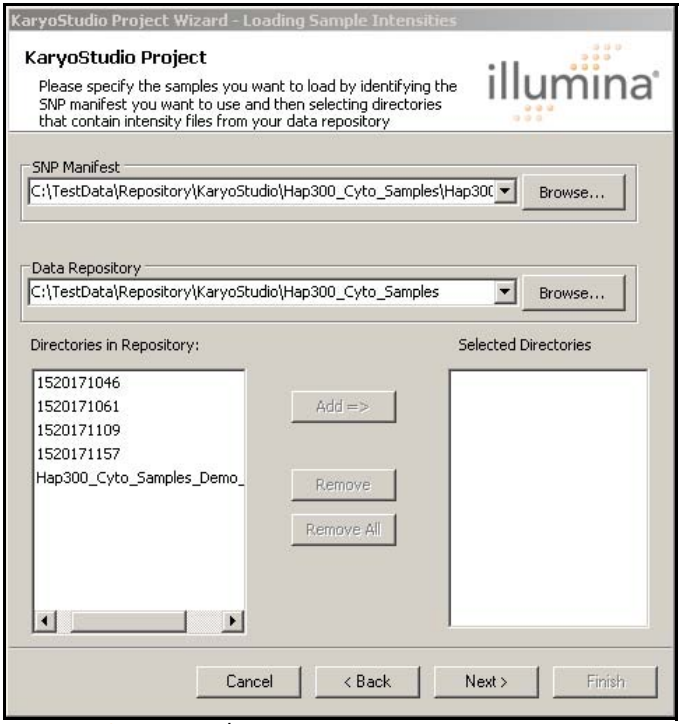

Figure 19 Wizard - Data Repository

4. Add data to this project by selecting items in the Directories in Repository area and clicking **Add**.

   The directories you selected appear in the Selected Directories area (Figure 20).

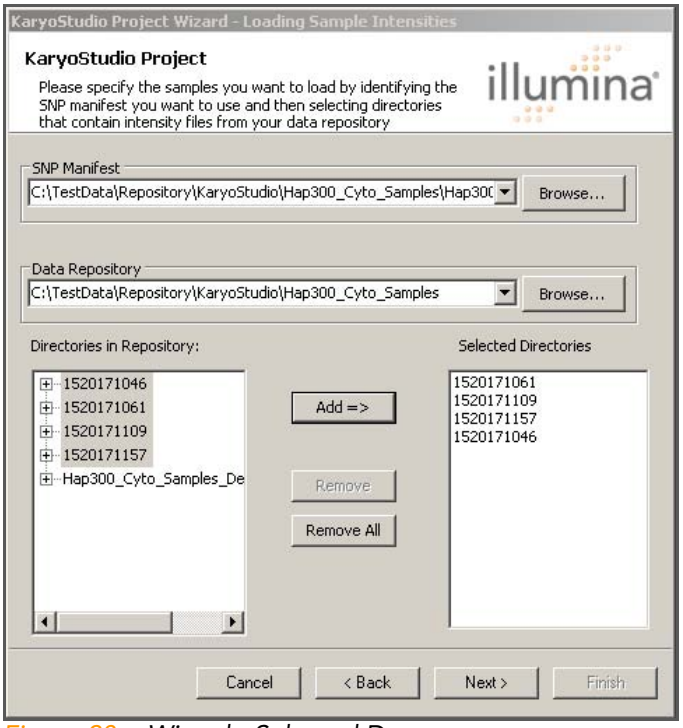

Figure 20 Wizard - Selected Data

5. Click **Next** to advance to the Cluster Positions screen (Figure 21).

KaryoStudio Project Wizard - Cluster Positions
KaryoStudio Project
If you have an existing cluster file that you want to import cluster positions from, enter it here. Otherwise, you can cluster the samples you've selected to determine cluster posit...
illumina
Cluster File
Browse...
Cancel
< Back
Next >
Finish

Figure 21 Wizard - Cluster Positions Screen

6. Browse to the cluster file you want to use with this project (Figure 22).

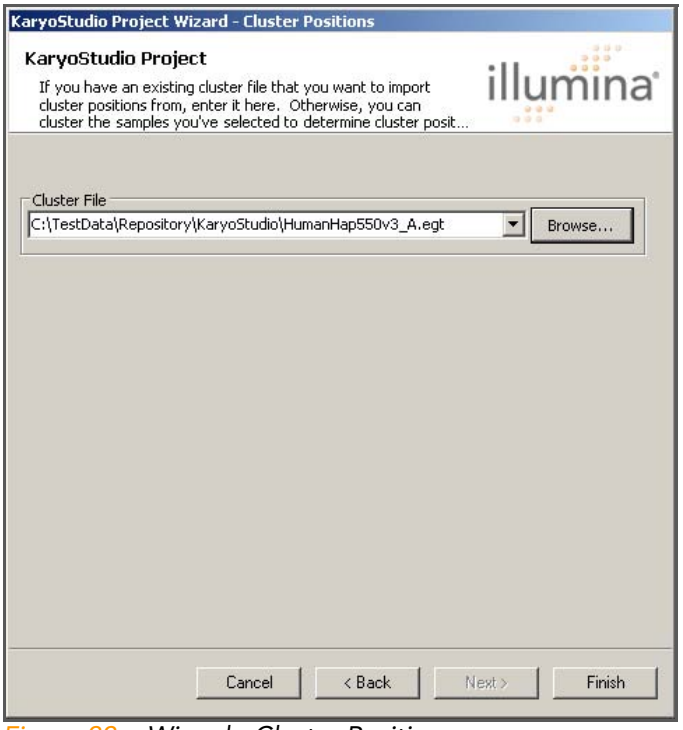
KaryoStudio Project Wizard - Cluster Positions
KaryoStudio Project
If you have an existing cluster file that you want to import cluster positions from, enter it here. Otherwise, you can cluster the samples you've selected to determine cluster posit...
illumina
Cluster File
C:\TestData\Repository\KaryoStudio\HumanHap550v3_A.egt
Browse...
Cancel
< Back
Next >
Finish

Figure 22 Wizard - Cluster Positions

7. Click **Finish**.

KaryoStudio displays a progress bar while it loads the intensity files, manifest file, and cluster files, and runs the cnvPartition algorithm on the data you chose to include in this project (Figure 23).

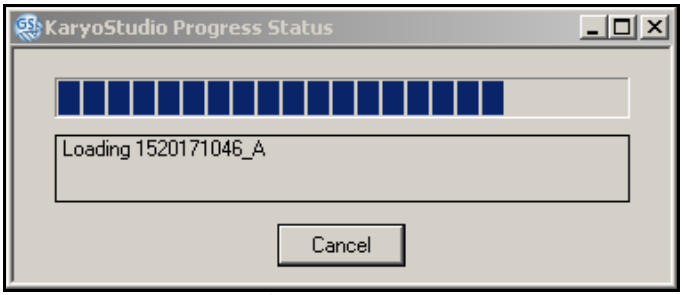

Figure 23 KaryoStudio Status Bar

NOTE

The amount of time it takes to create your project is directly related to the Infinium product you are using and the number of samples you are processing.

For example, it takes longer to load and analyze data from an Infinium HumanOmni1-Quad BeadChip than it does to load and analyze data from a HumanCytoSNP-12 BeadChip.

For more information about processing time, see the KaryoStudio System Information and Benchmark Performance Technical Note.

Once the data are finished processing and loading, KaryoStudio displays the new project (Figure 24).

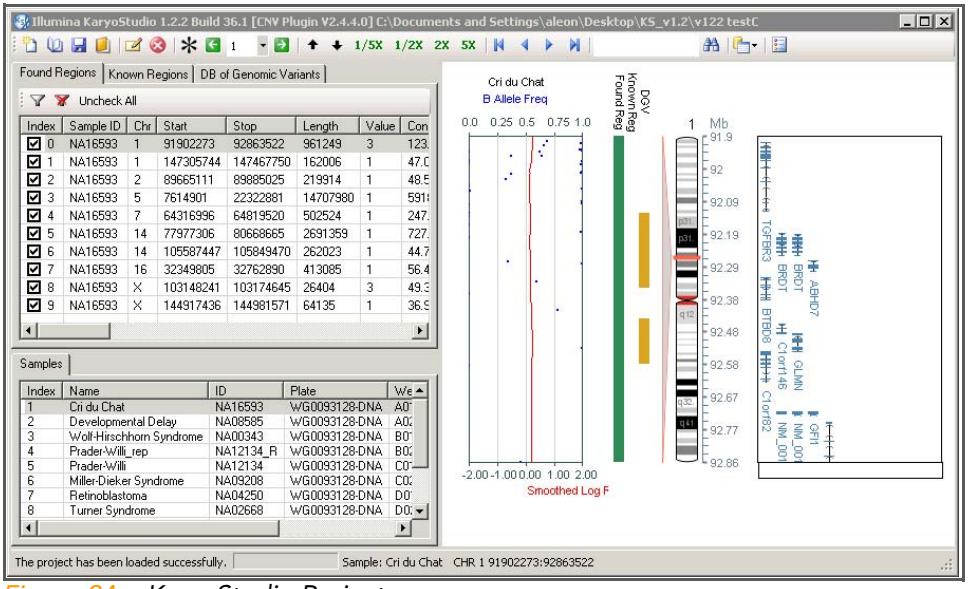
Illumina KaryoStudio 1.2.2 Build 36.1 [CNV Plugin V2.4.4.0] C:\Documents and Settings\aleon\Desktop\KS_v1.2\v122 testC
Found Regions
Known Regions
DB of Genomic Variants
Uncheck All
Index Sample ID Chr Start Stop Length Value
0 NA16593 1 91902273 92863522 961249 3
1 NA16593 1 147305744 147467750 162006 1
2 NA16593 2 89665111 89885025 219914 1
3 NA16593 5 7614901 22322881 14707980 1
4 NA16593 7 64316996 64819520 502524 1
5 NA16593 14 77977306 80668665 2691359 1
6 NA16593 14 105587447 105849470 262023 1
7 NA16593 16 32349805 32762890 413085 1
8 NA16593 X 103148241 103174645 26404 3
9 NA16593 X 144917436 144981571 64135 1
Samples
Index Name ID Plate
1 Cri du Chat NA16593 WG0093128-DNA
2 Developmental Delay NA08585 WG0093128-DNA
3 Wolf-Hirschhorn Syndrome NA00343 WG0093128-DNA
4 Prader-Willi_rep NA12134_R WG0093128-DNA
5 Prader-Willi NA12134 WG0093128-DNA
6 Miller-Dieker Syndrome NA09208 WG0093128-DNA
7 Retinoblastoma NA04250 WG0093128-DNA
8 Turner Syndrome NA02668 WG0093128-DNA
Cri du Chat
B Allele Freq
Known Reg
Found Reg
DGV
Mb
Smoothed Log R
The project has been loaded successfully.
Sample: Cri du Chat CHR 1 91902273:92863522

Figure 24 KaryoStudio Project

Opening an Existing Project

To open a project that was previously created and saved, perform the following steps:

1. From the KaryoStudio main window, click **Open an Existing Project**.
2. Browse to the location of the project you want to open and select **Open**.

   Your project is loaded into KaryoStudio, and the project data displays in the tables and chromosome browser.

Assessing the Quality of Your Data

After your samples are loaded and analyzed in KaryoStudio, the software automatically calculates a percent defects score and a LogRDev for each sample. These metrics are useful when determining whether there are any issues with a specific sample. The percent defect score and LogRDev are displayed in the lower left in the Samples Table.

The percent defects score is calculated by summing the length of all of the found regions within a particular sample of interest and dividing it by the length of the genome.

In control samples, such as non-constitutional samples, a percent defect score of less than 1% is expected. In most cases, the value is likely to be less than 0.5%. In samples that contain many aberrations, such as tumor samples, the percent defect score is expected to be much higher (in the 60-70% range). In addition, if there were any issues during the processing of your Infinium products, such as a misregistration or sample processing error, this score is expected to be abnormally high.

The LogRDev metric is a measure of the noise in the data, calculated as the standard deviation of the log R ratios for the SNPs on the autosomes. This metric is a simple indicator of the variation in the intensities measured for all markers from a particular sample. When analyzing relatively normal samples and following standard Illumina protocols for Infinium HD products, LogRDev is expected to be less than 0.3. For more information about interpreting LogRDev, see the Technical Note, *Evaluating Infinium Array Data for Copy Number Analysis*.

Illumina recommends analyzing control samples along with your experimental samples to ensure that there are minimal processing issues. This provides a good point of reference in the event that any issues do arise.

For additional information about how to QC your data, please refer to the KaryoStudio FAQs on the web, or contact Illumina Technical Support.

Chapter 3
User Interface

Topics

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Introduction

The KaryoStudio user interface provides tools for loading intensity files, browsing found regions, comparing found regions to known regions, and displaying them graphically.

Figure 25 shows KaryoStudio's screen configuration.

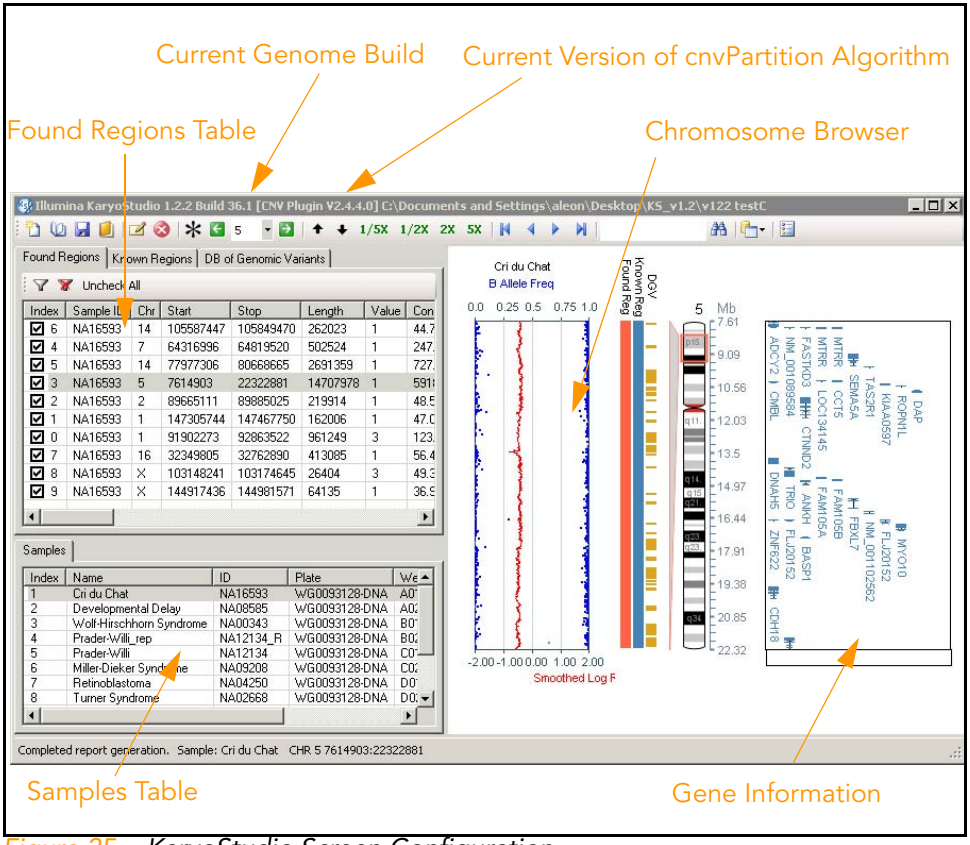


Figure 25 KaryoStudio Screen Configuration

Main Window Menus

Table 1 lists toolbar button options available from KaryoStudio's main window.

Table 1 KaryoStudio Toolbar Button Options

Toolbar Button	Name	Function
	Create New Project	Create a new project
	Open an Existing Project	Open a previously generated KaryoStudio project
	Save the Current Project	Save the current KaryoStudio project to your computer
	Close the Current Project	Close the current KaryoStudio project
	Write Reports to Project Directory	Generate a *.pdf report and save it to your computer
	Close Application	Close KaryoStudio
	Expand to Chromosome	Expands the current Chromosome Viewer view from selected found region to entire chromosome
	Previous Chromosome	Display previous chromosome in the Chromosome Viewer
	Select Chromosome	Select chromosome to display in the Chromosome Viewer
	Next Chromosome	Display next chromosome in the Chromosome Viewer
	Pan Up 1/4 Step	Adjust view slightly towards the beginning of the chromosome

Table 1 KaryoStudio Toolbar Button Options

Toolbar Button	Name	Function
	Pan Down 1/4 Step	Adjust view slightly towards the end of the chromosome
	Zoom in 5X	Adjust chromosome view to five times closer
	Zoom in 2X	Adjust chromosome view to two times closer
	Zoom out 2X	Expand chromosome view by two times
	Zoom out 5X	Expand chromosome view by five times
	First Aberration	Jump to the first found region in the Found Regions Table.
	Previous Aberration	Jump to the previous found region in the Found Regions Table.
	Next Aberration	Jump to the next found region in the Found Regions Table.
	Last Aberration	Jump to the last found region in the Found Regions Table.
	Settings	Adjust KaryoStudio settings for data plots and generating reports.
	Display Log	Display a log of all actions performed by KaryoStudio.

Table Window

The table window includes three tables:

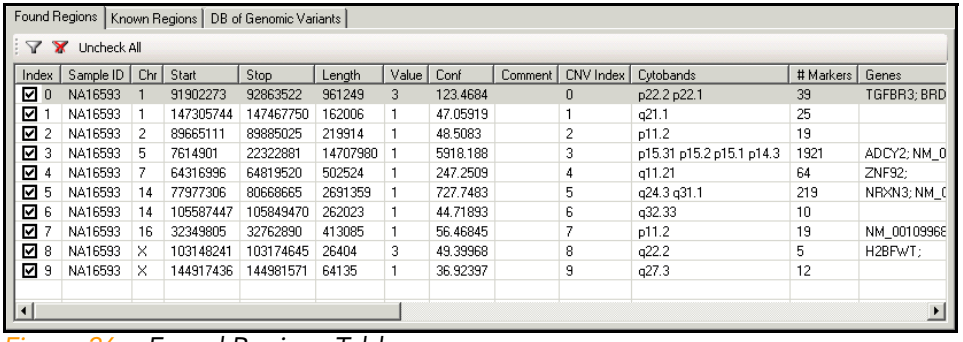
- Found Regions Table
- Known Regions Table
- Database of Genomic Variants

These tables are described in the following sections.

Found Regions Table

The Found Regions table (Figure 26) lists the regions passing the filter settings that were identified by the cnvPartition algorithm ("found regions"), and provides the following information:

- Sample name in which a region is found
- Chromosome on which a region appears
- Start and stop positions of region
- Length of region
- Value (Copy Number)
- Conf (Confidence Score)
- Comment (if any)
- CNV Index
- Cytobands
- Number of Markers
- Genes



Index	Sample ID	Chr	Start	Stop	Length	Value	Conf	Comment	CNV Index	Cytobands	# Markers	Genes
0	NA16593	1	91902273	92863522	961249	3	123.4684		0	p22.2 p22.1	39	TGFBR3; BRD
1	NA16593	1	147305744	147467750	162006	1	47.05919		1	q21.1	25	
2	NA16593	2	89665111	89885025	219914	1	48.5083		2	p11.2	19	
3	NA16593	5	7614901	22322881	14707980	1	5918.188		3	p15.31 p15.2 p15.1 p14.3	1921	ADCY2; NM_0
4	NA16593	7	64316996	64819520	502524	1	247.2509		4	q11.21	64	ZNF92;
5	NA16593	14	77977306	80668665	2691359	1	727.7483		5	q24.3 q31.1	219	NRXN3; NM_(
6	NA16593	14	105587447	105849470	262023	1	44.71893		6	q32.33	10	
7	NA16593	16	32349805	32762890	413085	1	56.46845		7	p11.2	19	NM_00109968
8	NA16593	X	103148241	103174645	26404	3	49.39968		8	q22.2	5	H2BFWT;
9	NA16593	X	144917436	144981571	64135	1	36.92397		9	q27.3	12	

Figure 26 Found Regions Table

The **Filter Table** and **Clear Filter** buttons allow you to filter the found regions that appear in the Found Regions table and track. For more information, see *Filtering Found Regions* on page 63.

The **Check All/Uncheck All** buttons allow you to include or exclude all displayed found regions from cytogenetics reports.

The columns in the Found Regions Table are listed and described in Table 2.

Table 2 Found Regions Table Columns

Column	Description
Index	Identifier given to each region identified by the cnvPartition algorithm across the entire KaryoStudio project (all samples)
Sample ID	Sample name • If you use a sample sheet to load your data, sample names are the names assigned to each sample in the sample sheet. • If you load your data without a sample sheet, sample names are barcodes.
Chr	Chromosome number of the found region
Start	Position of the first base of a found region
Stop	Position of the last base of a found region
Length	Length (in base pairs) of a found region
Value	Estimate of the physical copy number of a found region: • 0 indicates a homozygous deletion (loss of both copies) • 1 indicates a hemizygous deletion (loss of one copy) • 2 indicates a copy-neutral loss of heterozygosity (e.g., UPD or autozygosity) • 3 indicates a duplication (gain of one copy) • 4 indicates a copy number of 4 or above
Conf	Confidence score calculated for each found region by the cnvPartition algorithm. The confidence score is relative within a specific sample. A higher score represents higher confidence. Values of ~50 or higher tend to reflect regions with high confidence. Larger regions tend to have higher confidence scores.

Table 2 Found Regions Table Columns

Column	Description
Comment	User-entered comment
CNV Index	Number assigned to a found region within a specific sample. The range begins at 0.
Cytobands	List of cytobands in the found region
#Markers	Number of markers (SNPs or intensity-only probes) in a found region
Genes	RefSeq genes present within the found region

Changing your selection in the Found Regions table changes what is displayed in the Chromosome Browser to the right of the table.

Known Regions Table

The Known Regions table (Figure 27) lists all of the known regions that have been loaded into KaryoStudio.

Found Regions | Known Regions | DB of Genomic Variants

Index	Region	Chr	Start	End	Length	Disorder/Region
1	CytoGen1	1	0	27800000	27800000	1p36 Microdeletion
2	CytoGen2	1	16100000	20300000	4200000	Bartter3 (classic)
3	CytoGen3	1	16100000	56200000	40100000	Bartter4 (infantile with sensorineural deafness)
4	CytoGen4	1	60900000	68700000	7800000	NFIA Haploinsufficiency
5	CytoGen5	1	117600000	120700000	3100000	1p pericentromeric region
6	CytoGen6	1	142400000	148000000	5600000	1q21.1 Microdeletion with susceptibility for thrombocytopenia-absent radius (TAR)
7	CytoGen7	1	142400000	153300000	10900000	1q pericentromeric region
8	CytoGen8	1	174300000	178600000	4300000	"Short stature, pituitary and cerebellar defects, & small sella turcica "
9	CytoGen9	1	205300000	209500000	4200000	Van der Woude
10	CytoGen10	1	212100000	222100000	10000000	1q41-q42 Microdeletion/Fryns
11	CytoGen11	1	234600000	247249719	12649719	1q subtelomeric region
12	CytoGen12	1	241700000	247249719	5549719	1q44
13	CytoGen13	2	0	12800000	12800000	2p subtelomeric region
14	CytoGen14	2	12800000	17000000	4200000	Feingold syndrome
15	CytoGen15	2	38400000	41600000	3200000	Noonan4

Figure 27 Known Regions Table

In this table, Illumina provides information about commonly affected regions by default. However, you can also edit this table based on the requirements of your project.

The columns in the Known Regions Table are listed and described in Table 3.

Table 3 Known Regions Table Columns

Column	Description
Index	Numeric identifier assigned to known regions
Region	Specific name assigned to a known region
Chr	Chromosome on which the known region appears
Start	Position of the first base pair of the known region
End	Position of the last base pair of the known region
Length	Length of the known region
Disorder/Region	Name of the region, or the syndrome associated with chromosomal aberrations in this region

The default known regions file included with KaryoStudio is based mainly on affected chromosomal bands, so the base pair start and stop positions may be approximate.

Editing the Known Regions Table

Illumina has prepopulated the Known Regions table with a default list of regions of the genome that are of interest to cytogeneticists.

You can edit or add additional known regions files in order to better represent the regions of interest to your cytogenetics lab. You can also have multiple known regions files for different analysis workflows, or for use with different sample types.

To create a new Known Regions file, perform the following steps:

1. Create a new file using Microsoft Excel or a word processing program, or start with an existing Known Regions file.
2. Verify that the required column headers are included (see the bulleted list below).
3. Enter information in each row for each known region.
4. Save the file as a tab-delimited file (*.txt) in the KaryoStudio program directory.

   The default KaryoStudio program directory is: *C:\Program Files\Illumina\KaryoStudio*.

To edit a Known Regions file, perform the following steps:

1. Navigate to the KaryoStudio program directory.

   The default KaryoStudio program directory is: *C:\Program Files\Illumina\KaryoStudio*.
2. Open the known regions file to be edited (The default file is *KnownRegionsTable.txt*) in a text editing tool such as Microsoft Excel.

Any of the rows in this file can be edited; however, you cannot delete any columns as this will crash the software.

3. Make changes to the file as desired, by adding rows, deleting rows, or adjusting the parameters for each row.

   Some of the items you can adjust are:

   - Region
   - Disorder
   - Chr #
   - Start
   - End
   - Length
   - Length minus overlap

For example, if you would like to more precisely identify the start and stop positions for a particular region, you can adjust them in this file. You can also add new rows to this file. This may be useful if you would like to cross-match regions in future samples to regions your lab has tracked in the past.

4. After you edit the file, you must save your changes as a *.txt file.

Illumina recommends saving a copy of the original known regions table in the event that you would like to revert back to the default file that is originally provided with the software.

Database of Genomic Variants

The Database of Genomic Variants table (Figure 28) is a list of all of the CNV regions found in the latest build of the DGV. Information from this database is loaded into KaryoStudio as a text file and included for cross-matching purposes.

Found Regions | Known Regions | DB of Genomic Variants

Index	ID	Landmark	Chr	Start	End	Length	Variation Type	Locus Chr	Locus Start	L
1	Variation_0001	CTC-232B23	1	1083805	1283805	200000	CopyNumber	chr1	377	2
2	Variation_0002	RP1-283E3	1	1609516	1720123	110607	CopyNumber	chr1	377	2
3	Variation_0003	RP4-633I8	1	8668859	8812827	143968	CopyNumber	chr1	8668859	8
4	Variation_0004	RP11-219C24	1	13245159	13429749	184590	CopyNumber	chr1	12521755	1
5	Variation_0005	RP1-163M9	1	16880179	16998245	118066	CopyNumber	chr1	16612540	1
6	Variation_0006	RP3-423B22	1	37714745	37826968	112223	CopyNumber	chr1	37714745	3
7	Variation_0008	RP6-65F20	1	57288465	57408242	119777	CopyNumber	chr1	57288465	5
8	Variation_0009	RP11-131O15	1	67485262	67607550	122288	CopyNumber	chr1	67485262	6
9	Variation_0010	RP4-601K24	1	83274363	83403829	129466	CopyNumber	chr1	83101150	8
10	Variation_0011	RP11-335D10	1	95469800	95527429	57629	CopyNumber	chr1	95268437	9
11	Variation_0012	RP11-259N12	1	103956056	104113271	157215	CopyNumber	chr1	103762947	1
12	Variation_0013	RP4-580L15	1	113251567	113304968	53401	CopyNumber	chr1	112958658	1
13	Variation_0014	AL021069	1	172735904	172836399	100495	CopyNumber	chr1	172735904	1
14	Variation_0015	RP11-415M14	1	174297609	174457198	159589	CopyNumber	chr1	174297609	1
15	Variation_0016	RP5-1016N21	1	231322487	231474039	151552	CopyNumber	chr1	230661638	2

Figure 28 Database of Genomic Variants

The version of the DGV delivered with KaryoStudio v1.2 is http://projects.tcag.ca/variation/downloads/variation.hg18.v8.aug.2009.txt.

For information about updating KaryoStudio with the latest DGV build, see page 87.

The columns in the Database of Genomic Variants are listed and described in Table 4.

Table 4 Database of Genomic Variants Columns

Column	Description
Index	Number used as a simple identifier for regions
ID	Identifier assigned to a region by the Database of Genomic Variants; stays consistent over time
Landmark	BAC clone identifier for a specific region
Chr	Chromosome of a region
Start	Start position of a region
End	End position of a region
Length	Size of a region
Variation Type	Type of CNV
Locus Chr	General identifier that may contain multiple variation IDs
Locus Start	Start position of a locus region
Locus End	End position of a locus region
Reference	Publication reference showing where this region has been published
PubMed ID	Entrez PubMed ID of a publication linked to a specific region
Reference	Author of the study
Gain	Total number of patients in which a gain was seen
Loss	Total number of patients in which a loss was seen
Total Gain Loss	Total number of patients in which a gain or loss was seen
Sample Size	Total number of patients in the study

Samples Table

The Samples table (Figure 29) lists all of the samples you have loaded into KaryoStudio for the current project.

Samples

Index	Name	ID	Plate	Well	% Defects	Gender	LogRDev
1	Cri du Chat	NA16593	WG0093128-DNA	A01	0.65	Female	0.13
2	Developmental Delay	NA08585	WG0093128-DNA	A02	0.78	Male	0.11
3	Wolf-Hirschhorn Syndrome	NA00343	WG0093128-DNA	B01	0.87	Male	0.12
4	Prader-Willi_rep	NA12134_R	WG0093128-DNA	B02	0.05	Male	0.10
5	Prader-Willi	NA12134	WG0093128-DNA	C01	0.09	Male	0.10
6	Miller-Dieker Syndrome	NA09208	WG0093128-DNA	C02	0.19	Male	0.12
7	Retinoblastoma	NA04250	WG0093128-DNA	D01	0.06	Female	0.12
8	Turner Syndrome	NA02668	WG0093128-DNA	D02	4.67	Female	0.11
9	Cornelia de Lange	NA11165	WG0093128-DNA	E01	0.06	Male	0.12
10	Smith-Magenis Syndrome	NA18319	WG0093128-DNA	F01	0.17	Female	0.12
11	Down Syndrome	NA04592	WG0093128-DNA	G01	1.08	Female	0.13
12	Wilms Tumor	NA07427	WG0093128-DNA	H01	0.02	Male	0.12

Figure 29 Samples Table

When you select a sample in the Samples Table, the found regions for that sample appear in the Found Regions table above.

You can choose more than one sample by pressing and holding the **Ctrl** button on your keyboard while selecting multiple samples of interest. As you select each sample, the Found Regions table above is dynamically updated.

The columns in the Samples Table are listed and described in Table 5.

Table 5 Samples Table Columns

Column	Description
Index	Number assigned to the sample for sorting purposes
Name	Name or barcode of a sample from a BeadChip
ID	Barcode of a BeadChip
Plate	Location on the sample preparation plate
Well	Well on the sample preparation plate

Table 5 Samples Table Columns

Column	Description
% Defect	Score given to each sample based on the number of found regions. This value is the sum of the length of all found regions per sample divided by the length of the genome.
Gender	Gender of the sample, as provided in the sample sheet. KaryoStudio uses the gender information to correctly interpret found regions on the sex chromosomes.
LogRDev	Standard deviation of the log R ratios of the sample

Chromosome Browser

The Chromosome Browser includes representations of your data in the form of B allele frequency (genotyping information) represented by blue dots, and log R ratio (intensity information) represented by a grey line. The smoothed log R ratio is represented by a red line (Figure 30).

The B allele frequency for intensity-only probes is represented by light blue dots and is not used in copy number calculations.

In addition, there is an ideogram of the chromosome, found and known regions, information from the DGV (Database of Genomic) Variants, and gene information. You can adjust the display parameters from the **Settings** tab.

To hide or display data types in the plot, go to **Settings** | **Data Plot** and clear or select **Smoothed LogR**, **B Allele Freq**, or **LogR**.

The Log R Ratio axes are adjustable. By default, the axis range is -2.0 to 2.0. To adjust the Log R Ratio axis to fit the data, select **Settings** | **AutoScale** | **LogR Axis**.

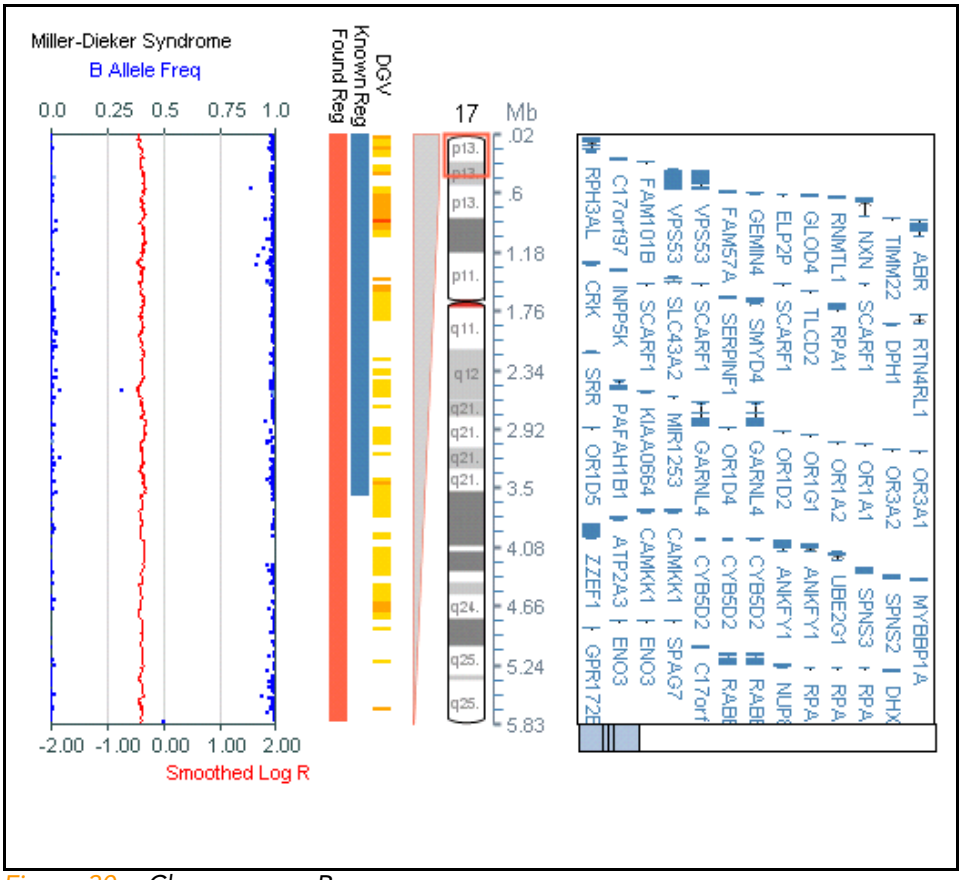


Figure 30 Chromosome Browser

Search Function

You can navigate to a particular location in the chromosome browser by entering a search term in the search field. Searches may be of the following types:

- **Chromosomal coordinate**

  Enter the chromosome number, a colon, and the start and stop coordinates separated by a hyphen.

  EXAMPLE: enter 1:1-2000000 to display the first 2Mb of chromosome 1.

- **Cytoband**

  Enter the location in ISCN notation.

  EXAMPLE: enter 1p22.1 or 1p22

- **Gene name**

  Entering text into the search box displays the first gene that matches the search term, starting with the chromosome currently displayed in the browser. To search a particular chromosome, start your search with the chromosome number and a colon.

  EXAMPLE: enter 6:VEGF to search for VEGFA rather than VEGFC on chromosome 4.

Navigating the Chromosome Browser

In addition to using the toolbar navigation buttons, you can zoom in and scroll by clicking and dragging the red box on the ideogram using your mouse.

To scroll, click and drag the red box up or down along the ideogram.

To zoom in our out, drag the top or bottom edge of the square up or down along the ideogram. The size of the box indicates the size of the chromosomal region displayed in the data plot.

If you place the cursor over a data point, a tooltip featuring the SNP name and coordinates is displayed (Figure 31).

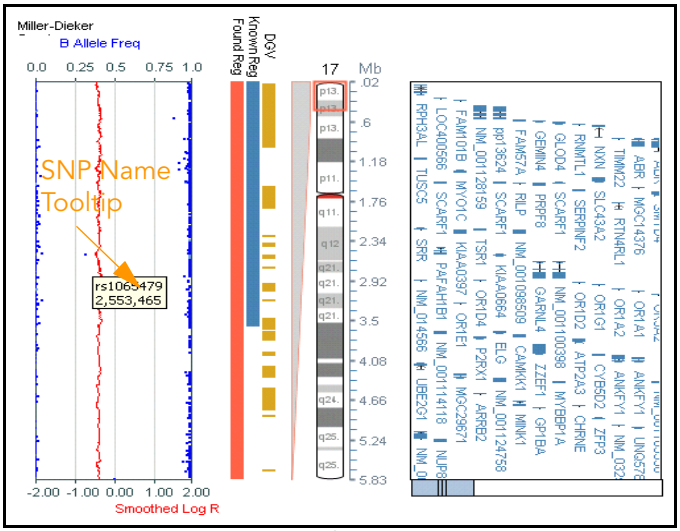


Figure 31 SNP Name Tooltip

If you place the cursor over a cytoband on the ideogram, a tooltip featuring the cytoband name is displayed (Figure 32).

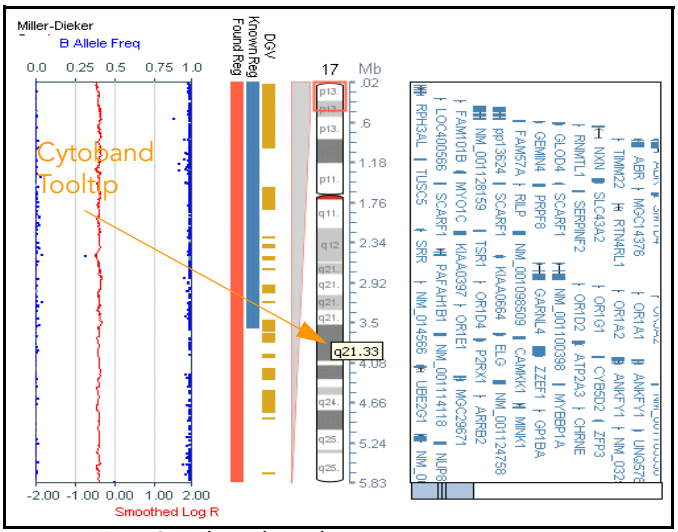


Figure 32 Cytoband Tooltip

If you place the cursor over a region found in the DGV track, a DGV tooltip is displayed (Figure 33).

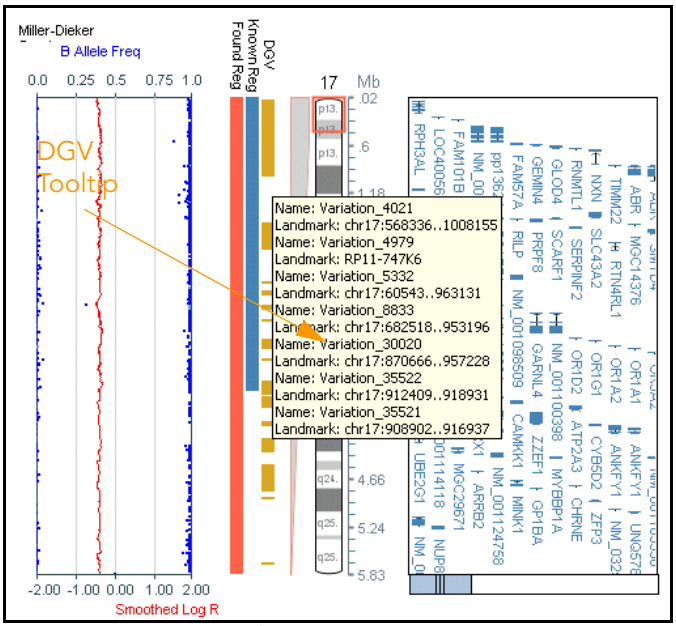


Figure 33 DGV Tooltip

The DGV track is multicolored. The colors indicate the numbers of DGV variation regions overlapping at a given point, as described below:

Table 6 DGV Track Colors

Color	Number of DGV Variation Regions Overlapping at a Given Point
light gold	1-2
orange	3-10
orange-red	11-20
red	>20

If you click anywhere along the ideogram, an alignment cursor displays as a dashed red line (Figure 34).

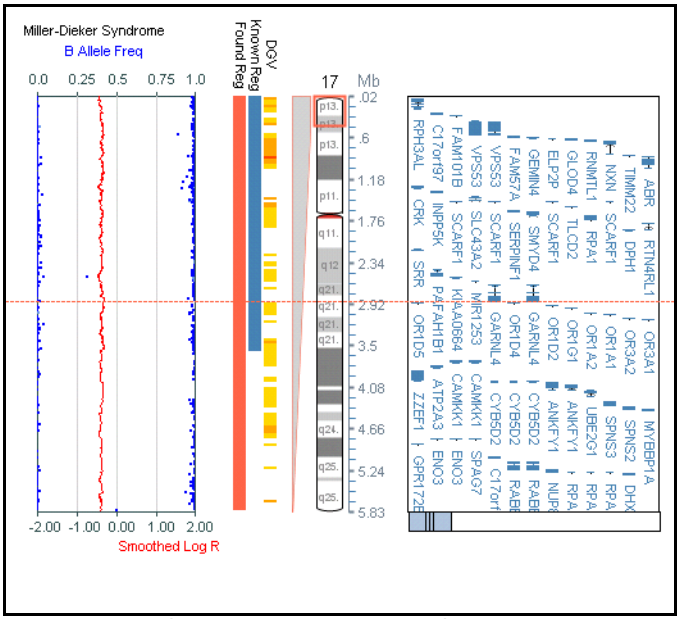


Figure 34 Chromosome Browser Alignment Cursor

In the gene display area to the right, gene names and transcript locations appear, as in the RefSeq database. To open RefSeq, right-click a gene name and select **Open RefSeq**.

The elements of the Chromosome Browser are listed and described in Table 7.

Table 7 Chromosome Browser Elements

Element	Description
Sample Name	Shows the sample being displayed
B allele frequency plots	Shows B allele frequency data for intensity-only markers in blue
Log R ratio plot	Shows smoothed log R ratio data in red or log R ratio data in grey
Found Regions	Track next to the chromosome showing a found region. Gains in copy number are indicated in green and losses are indicated in red.
Known Regions	Blue boxes represent known regions in this area. Directly correlates with the information in the Known Regions Table, populated from the Known Regions file currently selected in the Filter Settings table.
DGV	Track next to the chromosome shows correlation with information in the DGV (Database of Genomic Variants)
Chromosome	Vertical ideogram of the chromosome
Gene information	All available RefSeq gene information Note: For larger regions, such as whole chromosomes, the gene information appears very condensed. To refine the information use the zoom buttons.

Pseudoautosomal region (PAR) markers and found regions in the PAR are displayed as being on the X chromosome.

Karyotype View

The Karyotype View window displays a whole-genome view of the found regions for one or more samples. This view can be used to gain an initial appreciation for the variation found in a sample, or as a clickable visual table of contents for the found regions in a sample.

To use the karyotype view, perform the following steps:

1. In the Samples table, right-click a sample and select **Show Karyotype**. The Karyotype View window appears (Figure 35).

   All found regions are displayed in a track for each sample selected. Regions are color coded:

   - green = gain
   - red = loss
   - purple = copy-neutral event

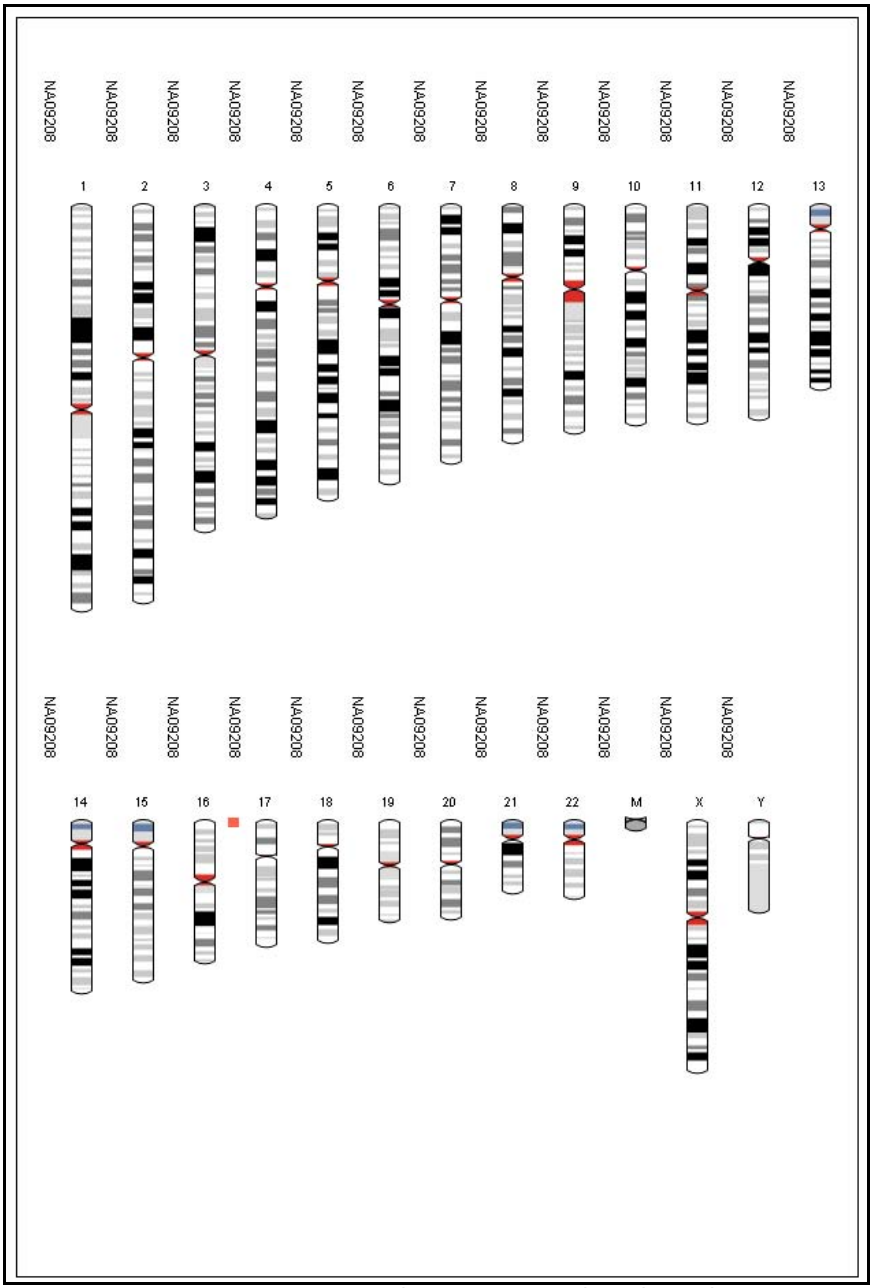


Figure 35 Karyotype View Window, One Sample Selected

2. To add or remove additional samples from the Karyotype View, press and hold the **Ctrl** key and click each sample in the Samples table.

   Information for all selected samples appears in the KaryoStudio View window (Figure 36).

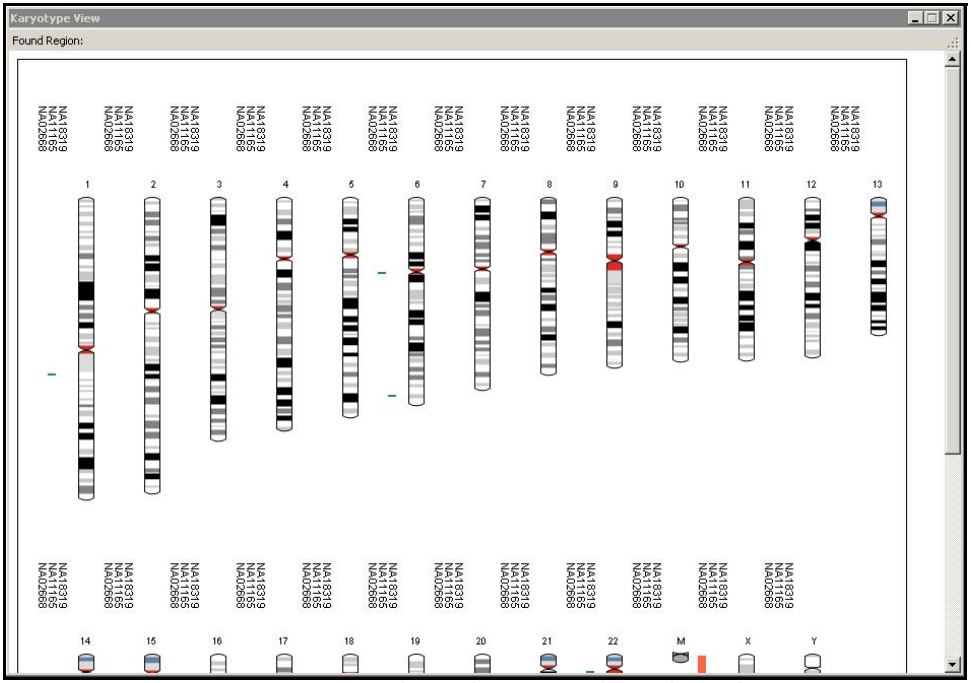


Figure 36 Karyotype View Window, Three Samples Selected

3. To display information about a found region in the info bar at the top of the window, hover over a found region.

   The information displayed includes: sample ID, chromosome, start and end coordinates, and copy number value (Figure 37).

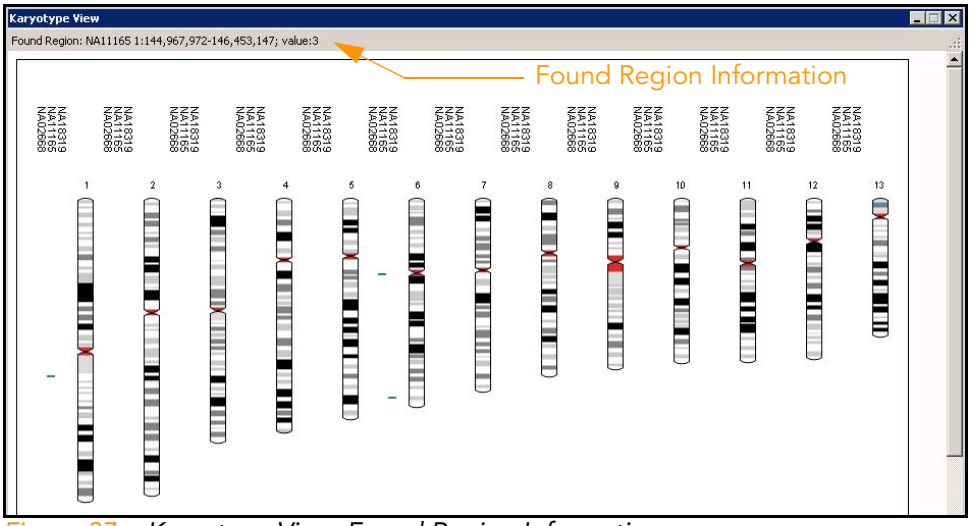


Figure 37 Karyotype View, Found Region Information

4. To display a region in the chromosome browser, click a found region.

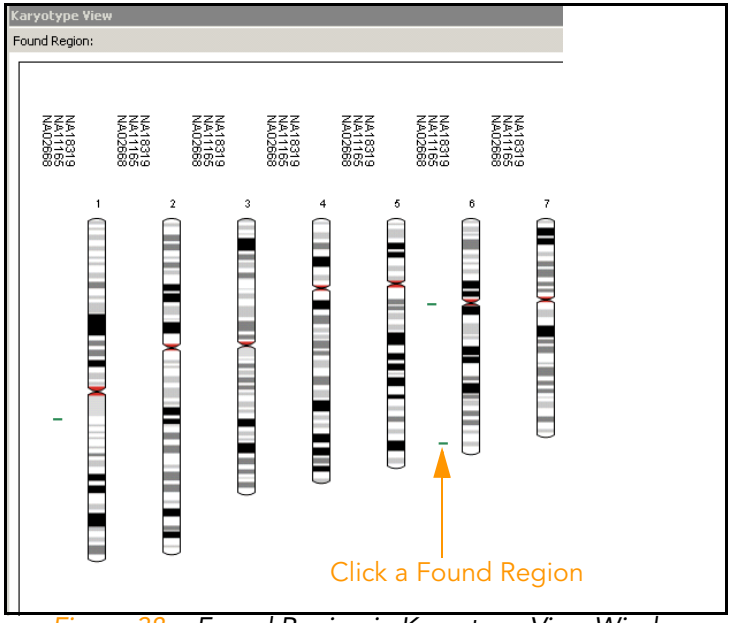


Figure 38 Found Region in Karyotype View Window

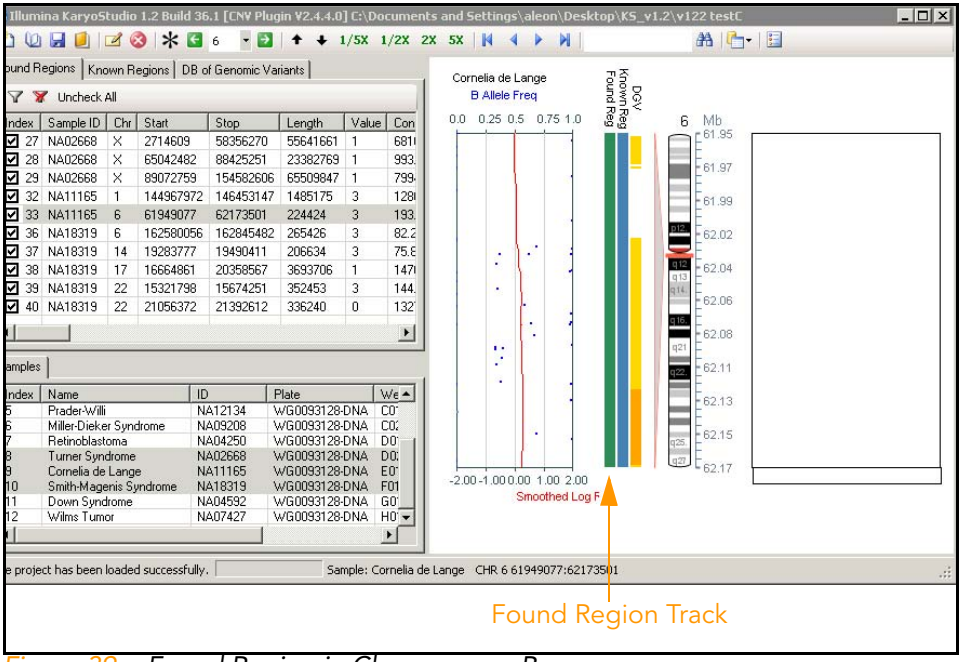


Figure 39 Found Region in Chromosome Browser

Duo and Trio View

Data plots from multiple samples can be displayed simultaneously in the chromosome browser. Duo and trio view can be used to analyze, for example, a parent-parent-child trio, a control sample, or different display settings for two plots from the same sample.

To use the duo and trio view, perform the following steps:

1. In the Samples table, select the samples you want to display.

2. To add a second data plot, select **Settings** | **Trio View** | **Data Plot 2** | and select a sample from the dropdown menu (Figure 40).

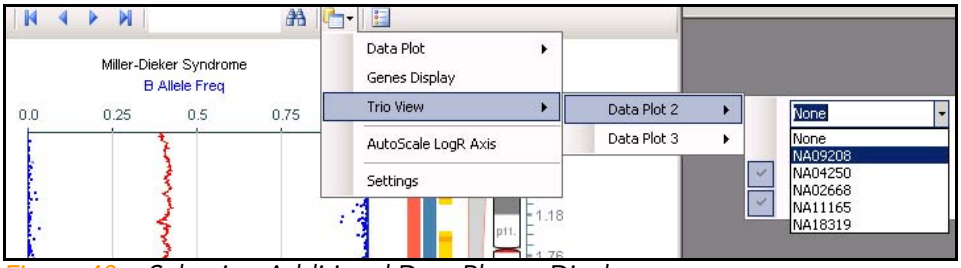


Figure 40 Selecting Additional Data Plot to Display

The second data plot appears in chromosome browser to the right of the original data plot (Figure 41).

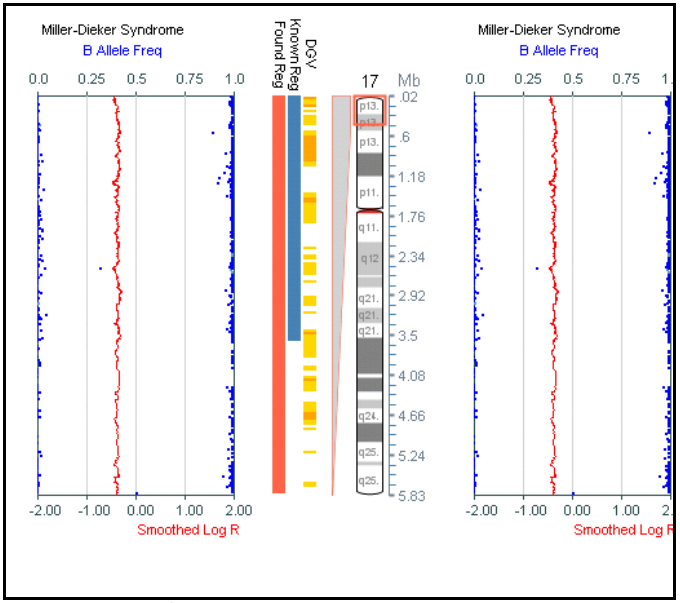


Figure 41 Chromosome Browser, Duo View

3. [Optional] To add a third data plot, select **Settings** | **Trio View** | **Data Plot 3** | and select a sample from the dropdown menu.

   The third data plot appears in chromosome browser to the right of the second data plot (Figure 42).

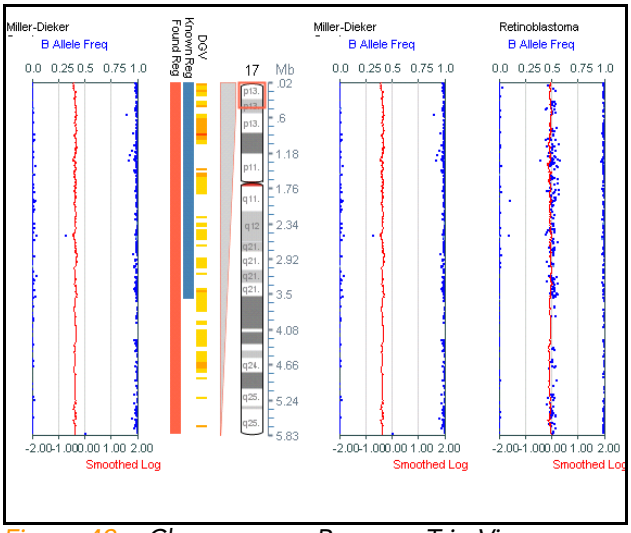


Figure 42 Chromosome Browser, Trio View

To select additional settings for each data plot, go to **Settings** | **Trio view** | **Data Plot**, and select or clear the **Log R**, **B Allele Freq**, and/or **Smoothed Log R** checkboxes (Figure 43).

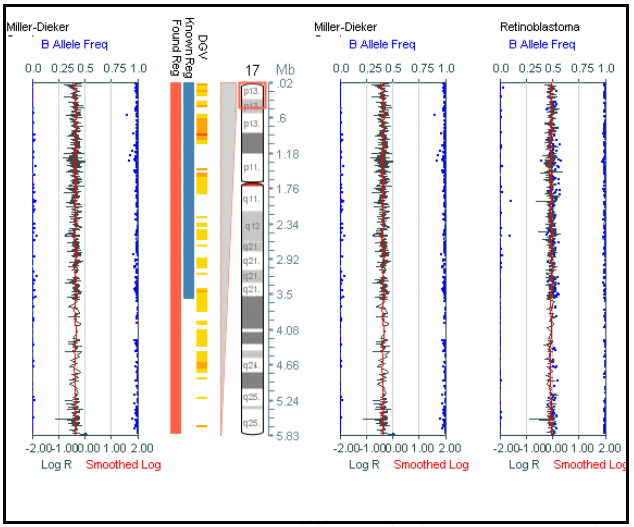


Figure 43 Trio View, Additional Plot Settings Selected

4. To return to a single-plot view with the genes display visible, select **Settings** | **Genes Display** (Figure 44).

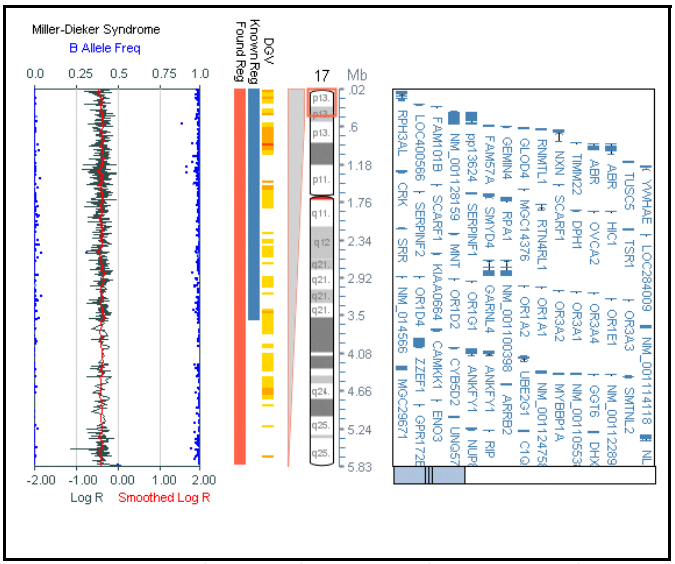


Figure 44 Single Sample View with Gene Display

Log Window

The Log window displays information about the projects and tracks for this analysis as they are being loaded into the software.

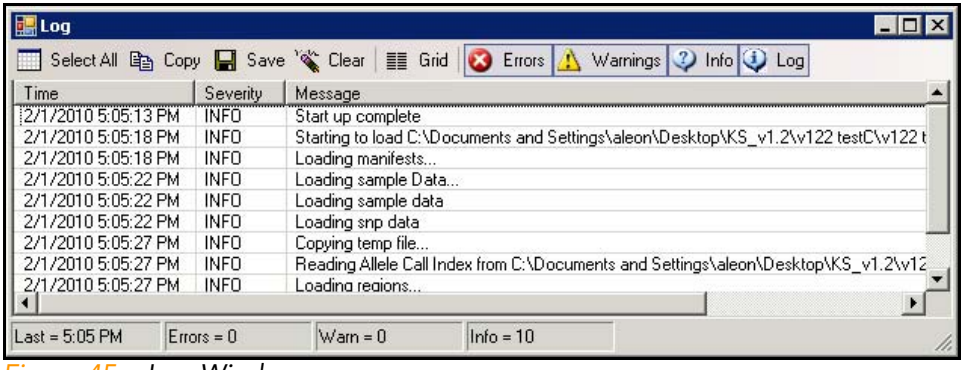


Figure 45 Log Window

The elements of the Log window are listed and described in Table 8.

Table 8 Log Window Elements

Element	Description	Toolbar Button (if used)
Select All	Selects all log entries	
Copy	Copies log entries to the clipboard	
Save	Saves all log entries	
Clear	Clears all log entries	
Grid	Toggles the grid on and off	
Time	Displays the time the log entry was generated	
Severity	Displays the severity of the log entry	
Message	Displays the text description of the log entry	
Source	Displays the source of the log entry	

Chapter 4

Analyzing Found Regions

Topics

62 Introduction
63 Filtering Found Regions
65 Displaying Found Regions
67 Scrolling Through Found Regions
68 Sorting Found Regions
70 Adding or Editing Comments
70 Adjusting Found Region Parameters
82 Exporting Found Regions

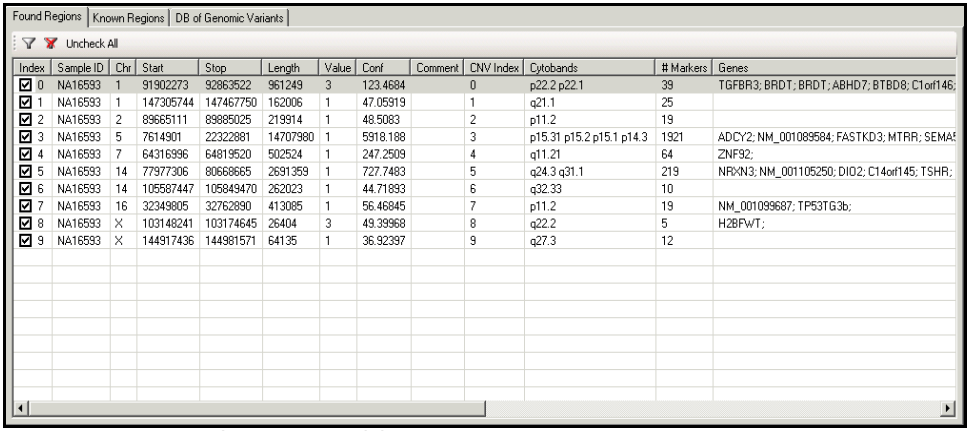
Introduction

KaryoStudio includes Illumina's cnvPartition algorithm, which is designed to scan whole-genome Infinium data for intensity changes resulting from changes in copy number. Once you have completed entering information into the Project Wizard, your project data is automatically scanned using the cnvPartition algorithm.

Detected aberrations are displayed in the Found Regions table. Included in this table is information about each aberration including:

- the sample in which it was found
- the chromosome start and stop position
- its size (length in bases)
- an estimate of the copy number (**Value** column)
- a confidence value (relative score)
- its cytobands
- the number of SNPs it contains
- the genes present in the found region

Once you have created a project, all detected regions are displayed in the Found Regions Table and listed in the order in which they were found in each sample (Figure 46).


Found Regions | Known Regions | DB of Genomic Variants

Uncheck All

Index	Sample ID	Chr	Start	Stop	Length	Value	Conf	Comment	CNV Index	Cytobands	# Markers	Genes
0	NA16593	1	91902273	92863522	961249	3	123.4684		0	p22.2 p22.1	39	TGFBR3; BRDT; BRDT; ABHD7; BTBD8; C1orf146;
1	NA16593	1	147305744	147467750	162006	1	47.05919		1	q21.1	25	
2	NA16593	2	89665111	89885025	219914	1	48.5083		2	p11.2	19	
3	NA16593	5	7614901	22322881	14707980	1	5918.188		3	p15.31 p15.2 p15.1 p14.3	1921	ADCY2; NM_001089584; FASTKD3; MTRR; SEMA5
4	NA16593	7	64316996	64819520	502524	1	247.2509		4	q11.21	64	ZNF92;
5	NA16593	14	77977306	80668665	2691359	1	727.7483		5	q24.3 q31.1	219	NRXN3; NM_001105250; DIO2; C14orf145; TSHR;
6	NA16593	14	105587447	105849470	262023	1	44.71893		6	q32.33	10	
7	NA16593	16	32349805	32762890	413085	1	56.46845		7	p11.2	19	NM_001099687; TP53TG3b;
8	NA16593	X	103148241	103174645	26404	3	49.39968		8	q22.2	5	H2BFWT;
9	NA16593	X	144917436	144981571	64135	1	36.92397		9	q27.3	12	

Figure 46 Found Regions Table

This chapter describes how to view and analyze the Found Regions in your data.

Filtering Found Regions

When a project is created, the cnvPartition algorithm discovers all regions of aberrant copy number based on the cnvPartition configuration settings.

By default, cnvPartition identifies all regions with confidence value greater than 35 and all copy-neutral LOH regions larger than 1Mb. However, these confidence and size thresholds are likely not stringent enough for most uses.

More specific filtering parameters for size, number of markers, and confidence threshold can be entered in KaryoStudio. After entering the desired parameters, only regions of potential interest which meet these criteria are displayed in the Found Regions Table and chromosome browser.

For information about adjusting the cnvPartition configuration file, see the cnvPartition documentation.

After a project is created, filter settings are applied to determine which regions display in the Found Regions Table. Default settings are applied to a new project, but these parameters can be changed easily.

To change the filter settings, perform the following steps:

1. Click **Filter Table**.

   The FoundRegionsFilterForm appears (Figure 47).

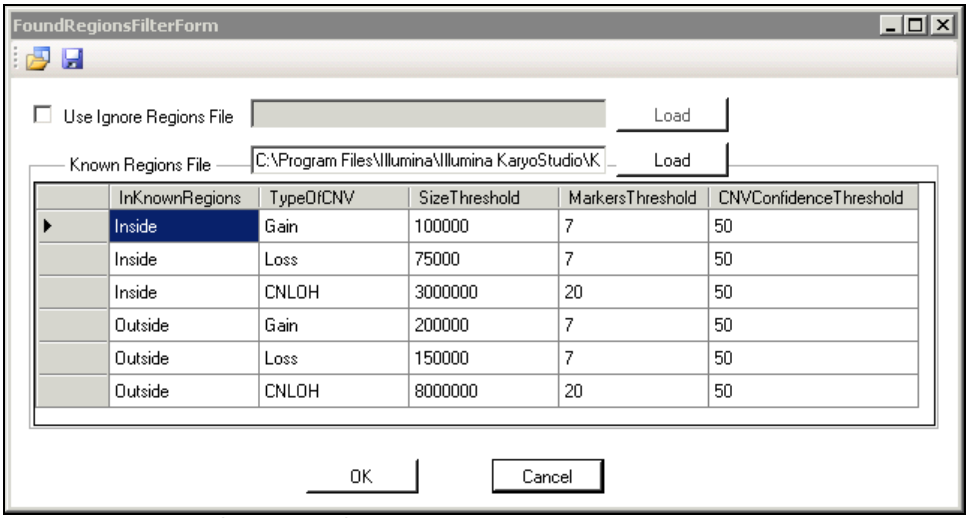


Figure 47 FoundRegionsFilterForm

The Known Regions File to be used in the project is listed in the second text field.

2. To change the Known Regions File, click **Load** and browse to the file you would like to use instead.

   See Appendix C of this document for information about formatting a Known Regions File.

   Different classes of found regions can be assigned different filter settings:

   - Settings for regions that at least partially overlap with regions defined in the selected Known Regions File are labeled **Inside** in the InKnownRegions column.
   - Settings for regions that do not overlap with regions defined in the selected Known Regions File are labeled **Outside** in the InKnownRegions column.
   - Settings for Gains, Losses, and Copy-Neutral events (CNLOH) can be set independently in the column **TypeOfCNV**.

3. [Optional] You can define found regions from which data should never be displayed by selecting the **Use Ignore Regions File** checkbox and loading a file.

In order to be ignored, a Found Region must fall completely within an Ignored Region.

This feature has many potential uses, such as ignoring common polymorphic CNVs or constraining analysis to a portion of the genome. For example, you could ignore all chromosomes except 21 to analyze only aberrations on chromosome 21.

See Appendix D of this document for more information about formatting an Ignored Regions File.

4. To save the settings in the FoundRegionsFilterForm, click **Save Filter Settings**.
5. To load settings from a file into the FoundRegionsFilterForm, click **Load Filter Settings** and browse to the settings file of interest.
6. Click **OK** to apply the new filter settings.

Displaying Found Regions

Notice that when you select a found region, the entire region displays in the chromosome browser (Figure 48). By default, genotyping information is displayed as B-allele frequency (blue dots) and intensity information is displayed as smoothed log R ratio (red line).

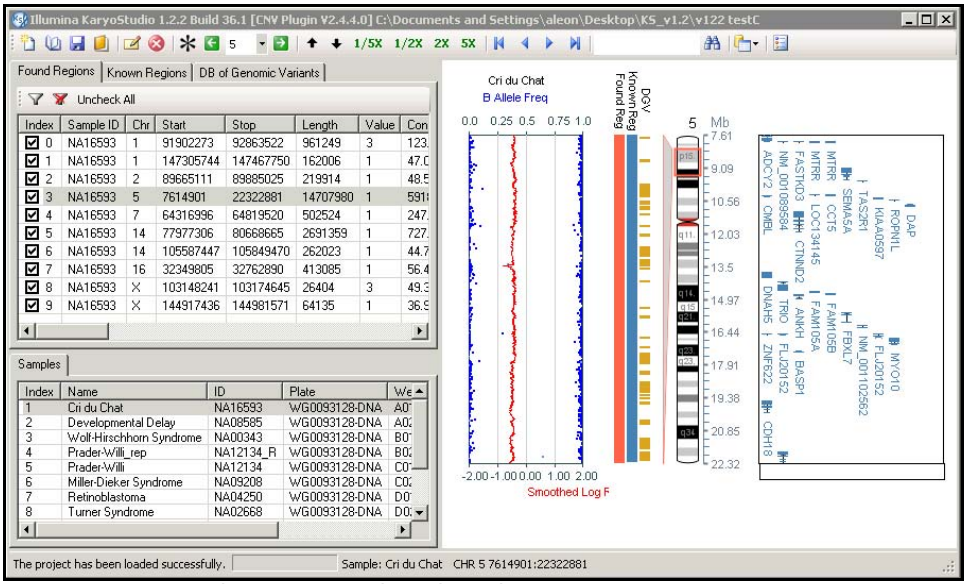

Illumina KaryoStudio 1.2.2 Build 36.1 [CNV Plugin V2.4.4.0] C:\Documents and Settings\aleon\Desktop\KS_v1.2\v122 testC
Found Regions
Known Regions
DB of Genomic Variants
Uncheck All
Index Sample ID Chr Start Stop Length Value
0 NA16593 1 91902273 92863522 961249 3
1 NA16593 1 147305744 147467750 162006 1
2 NA16593 2 89665111 89885025 219914 1
3 NA16593 5 7614901 22322881 14707980 1
4 NA16593 7 64316996 64819520 502524 1
5 NA16593 14 77977306 80668665 2691359 1
6 NA16593 14 105587447 105849470 262023 1
7 NA16593 16 32349805 32762890 413085 1
8 NA16593 X 103148241 103174645 26404 3
9 NA16593 X 144917436 144981571 64135 1
Samples
Index Name ID Plate
1 Cri du Chat NA16593 WG0093128-DNA
2 Developmental Delay NA08585 WG0093128-DNA
3 Wolf-Hirschhorn Syndrome NA00343 WG0093128-DNA
4 Prader-Willi_rep NA12134_R WG0093128-DNA
5 Prader-Willi NA12134 WG0093128-DNA
6 Miller-Dieker Syndrome NA09208 WG0093128-DNA
7 Retinoblastoma NA04250 WG0093128-DNA
8 Turner Syndrome NA02668 WG0093128-DNA
Cri du Chat
B Allele Freq
Known Reg
Found Reg
DGV
5 Mb
Smoothed Log R
The project has been loaded successfully.
Sample: Cri du Chat CHR 5 7614901:22322881

Figure 48 Found Region Displayed in Chromosome Browser

If you want to view the selected aberration in relation to the chromosome in which it appears, click **Expand to Chromosome**.

Intensity data for the whole chromosome is displayed (Figure 49).

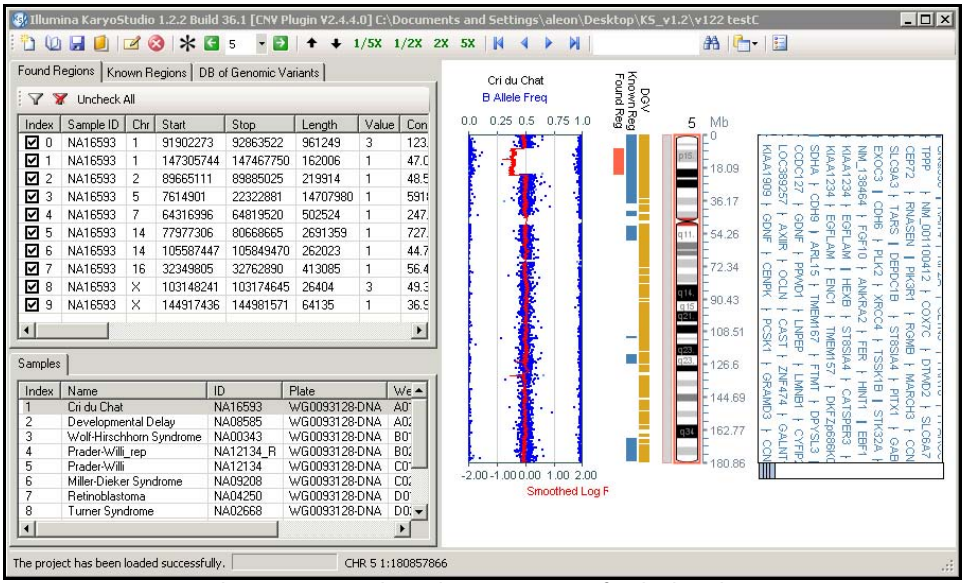


Figure 49 Found Region Displayed in Context of Whole Chromosome

Scrolling Through Found Regions

Once your data has loaded, you can view each found region one by one. There are multiple ways to select data to display in the chromosome browser:

- Click a found region to display it in the chromosome browser.
- Use the blue arrow buttons to move up or down in the Found Regions table.
- Use your mouse wheel to scroll up or down the Found Regions table.

Found regions are only displayed for the sample(s) selected in the Samples table. You can choose multiple samples by Ctrl-clicking or Shift-clicking multiple rows in the Samples table.

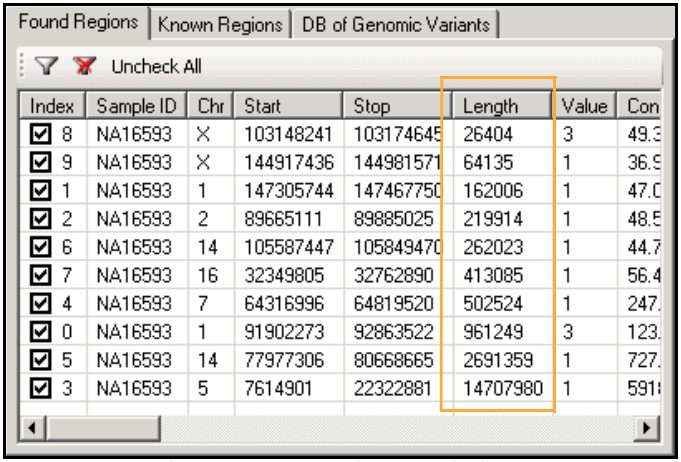
Sorting Found Regions

In addition to clicking and scrolling in the Found Regions table, you can sort data based on whichever column you like. To sort data, click a column header.

NOTE

If your found regions table contains hundreds of aberrations, it may take some time for KaryoStudio to sort the regions by column header.

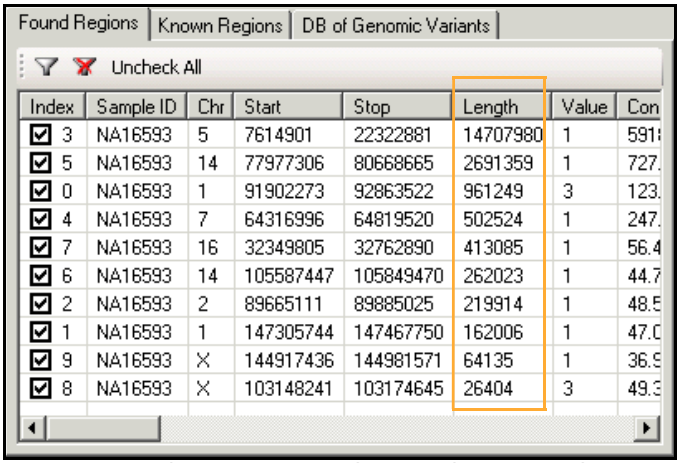
EXAMPLE 1: Click the column header **Length**, which represents the number of bases of an aberration. The aberrations listed in this table are now sorted from shortest to longest (Figure 50).



Index	Sample ID	Chr	Start	Stop	Length	Value	Con
8	NA16593	X	103148241	103174645	26404	3	49.3
9	NA16593	X	144917436	144981571	64135	1	36.9
1	NA16593	1	147305744	147467750	162006	1	47.0
2	NA16593	2	89665111	89885025	219914	1	48.5
6	NA16593	14	105587447	105849470	262023	1	44.7
7	NA16593	16	32349805	32762890	413085	1	56.4
4	NA16593	7	64316996	64819520	502524	1	247.
0	NA16593	1	91902273	92863522	961249	3	123.
5	NA16593	14	77977306	80668665	2691359	1	727.
3	NA16593	5	7614901	22322881	14707980	1	591

Figure 50 Aberration Length Sorted Short to Long

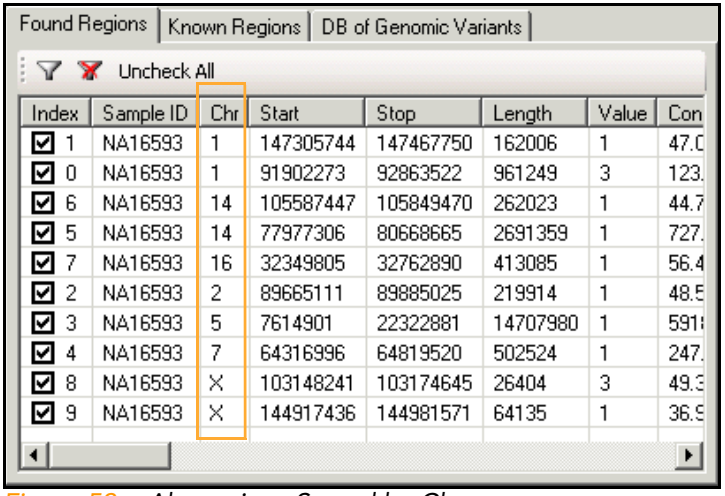
Click **Length** again to reverse the sort order. The aberrations now display from longest to shortest (Figure 51).



Index	Sample ID	Chr	Start	Stop	Length	Value	Con
3	NA16593	5	7614901	22322881	14707980	1	591
5	NA16593	14	77977306	80668665	2691359	1	727.
0	NA16593	1	91902273	92863522	961249	3	123.
4	NA16593	7	64316996	64819520	502524	1	247.
7	NA16593	16	32349805	32762890	413085	1	56.4
6	NA16593	14	105587447	105849470	262023	1	44.7
2	NA16593	2	89665111	89885025	219914	1	48.5
1	NA16593	1	147305744	147467750	162006	1	47.0
9	NA16593	X	144917436	144981571	64135	1	36.9
8	NA16593	X	103148241	103174645	26404	3	49.3

Figure 51 Aberration Length Sorted Long to Short

EXAMPLE 2: Click **Chr** to sort aberrations based on the number of the chromosome on which they occur. Sorting by chromosome is useful if you want to view only aberrations occurring on a certain chromosome (Figure 52).



Index	Sample ID	Chr	Start	Stop	Length	Value	Con
1	NA16593	1	147305744	147467750	162006	1	47.0
0	NA16593	1	91902273	92863522	961249	3	123.
6	NA16593	14	105587447	105849470	262023	1	44.7
5	NA16593	14	77977306	80668665	2691359	1	727.
7	NA16593	16	32349805	32762890	413085	1	56.4
2	NA16593	2	89665111	89885025	219914	1	48.5
3	NA16593	5	7614901	22322881	14707980	1	591
4	NA16593	7	64316996	64819520	502524	1	247.
8	NA16593	X	103148241	103174645	26404	3	49.3
9	NA16593	X	144917436	144981571	64135	1	36.9

Figure 52 Aberrations Sorted by Chromosome

NOTE

When you choose to sort, the data for all samples are sorted together. Therefore, found regions from multiple samples may be intermixed.

Adding or Editing Comments

To add comments or edit existing comments for a found region, enter or change a comment in the **Comments** field.

For example, you can type a note for a colleague suggesting that he or she follows up on this region later. You may also want to enter additional information obtained from your cross-matching results. The comments you enter are included in the Comments section of the *.pdf reports you generate.

Adjusting Found Region Parameters

Though it is not generally necessary to do so, KaryoStudio offers the ability to edit some parameters of a found region such as confidence score and estimated copy number value. KaryoStudio also gives you the option to enter comments for a found region. In addition, if necessary, you can edit the start and stop positions of a found region.

Editing Confidence Score or Estimated Copy Number

To adjust the confidence score or estimated copy number value of a found region, perform the following steps:

1. Right-click a region of interest in the Found Regions table.
   The context menu appears (Figure 53).

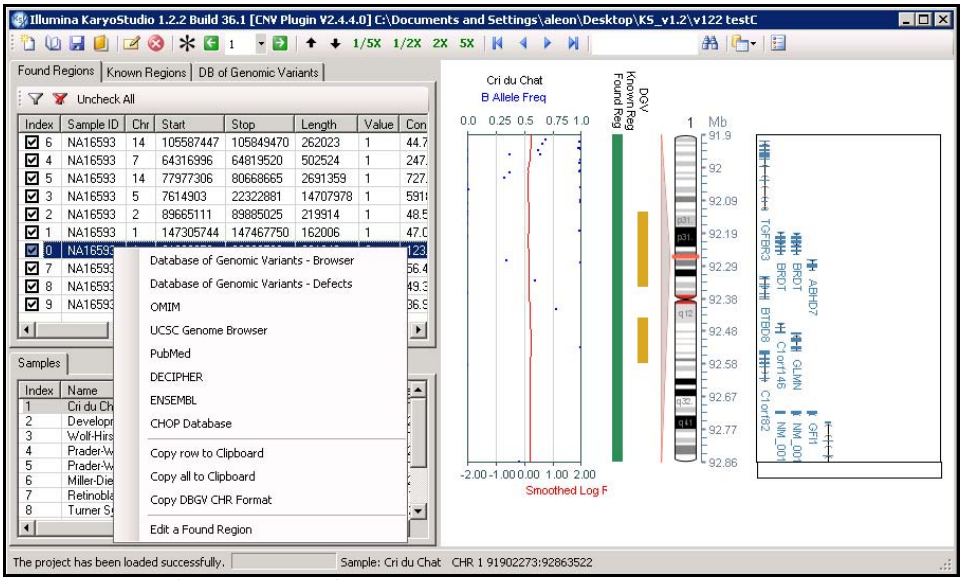


Figure 53 Editing a Found Region

2. Select **Edit a Found Region** from the context menu. The FormSettings screen appears (Figure 54).

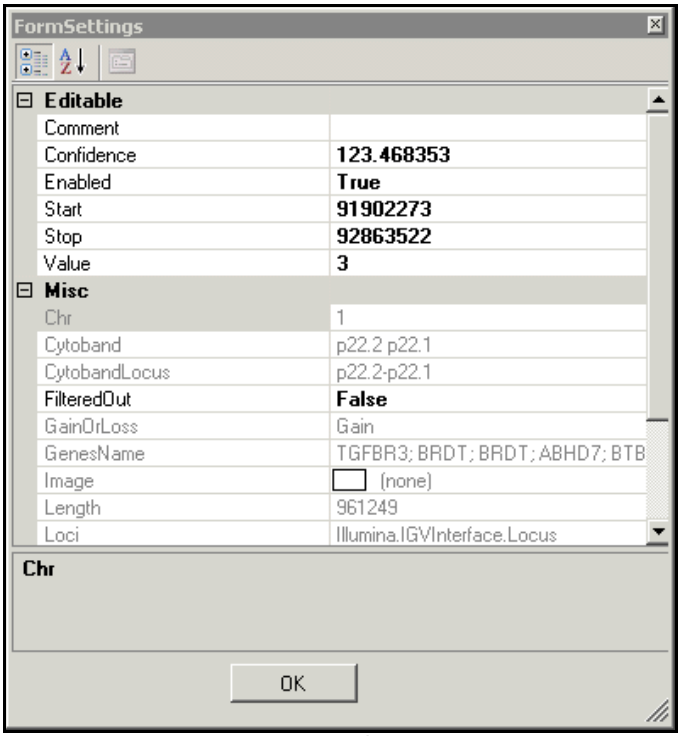


Figure 54 Form Settings Dialog Box

3. Do one of the following:
   - If you would like to change the confidence value for this region, adjust the **Confidence** score.

Although it is not recommend to change the confidence score of a found region, you can change it to 0 if you want to exclude this found region from analysis.

   - Adjust the **Estimated Copy Number Value** if, on visual inspection, you feel that cnvPartition has not accurately estimated the copy number of the found region.

The parameters in the Misc area cannot be adjusted.

Editing Start and Stop Positions

You can verify the accuracy of the cnvPartition algorithm by examining the start and stop positions of the found region. In some cases you may want to adjust start and stop positions based on visual inspection, though it is not common to do so. KaryoStudio also allows you to optionally adjust the information in the Found Regions table.

If you edit the start and stop positions of a found region, the original positions identified by the cnvPartition algorithm are not stored with the project.

The confidence score, number of SNPs, and genes do not update after you have edited a region.

If you need to retain the original start and stop positions for any reason, copy the Found Regions table before you load a project and save it as a separate file, or save the project on your computer with a different file name.

To adjust the start and stop positions of a found region of interest:

1. In the Found Regions table, select a region of interest (Figure 55).

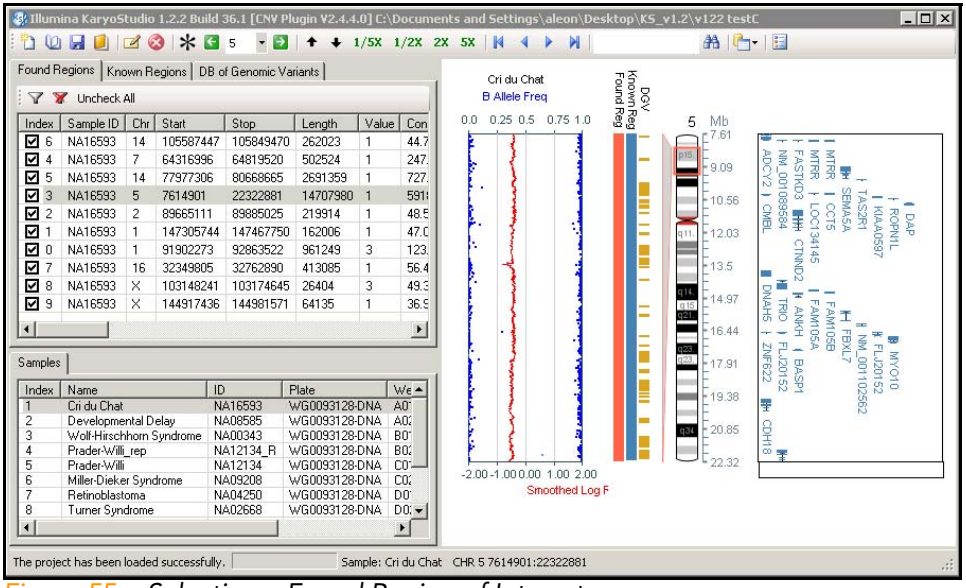


Figure 55 Selecting a Found Region of Interest

KaryoStudio displays your found region within the chromosome browser.

2. To expand the view so that you can see additional data adjacent to the beginning of the found region, click **Pan Up** (Figure 56).

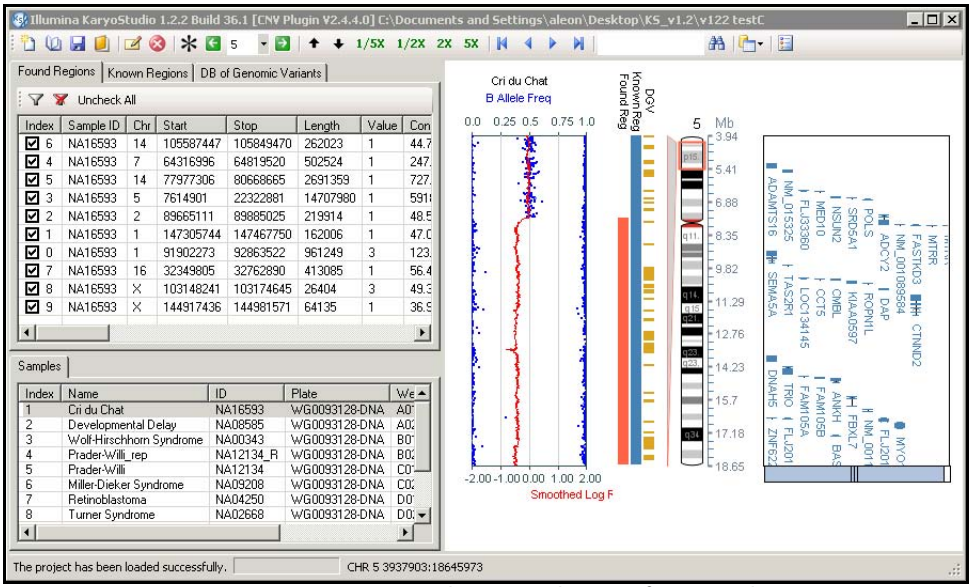


Figure 56 Panning Up to Examine the Boundaries of a Found Region

Notice that the red track indicating the found region has shifted lower on the screen.

To expand the view so that you can see additional data adjacent to the end of the found region, click **Pan Down** (Figure 57).

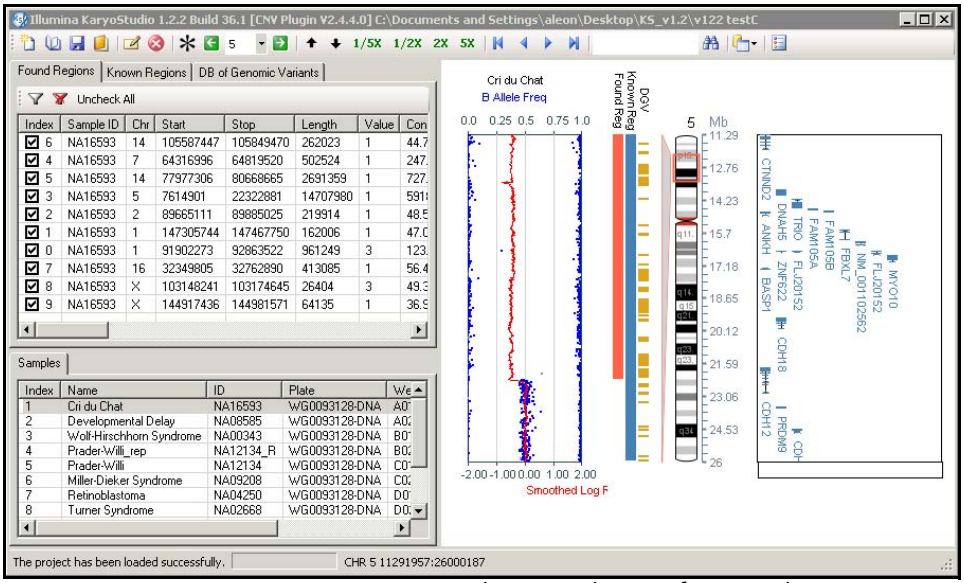

Illumina KaryoStudio 1.2.2 Build 36.1 [CNV Plugin V2.4.4.0] C:\Documents and Settings\aleon\Desktop\KS_v1.2\v122 testC
1/5X 1/2X 2X 5X
Found Regions
Known Regions
DB of Genomic Variants
Uncheck All
Index Sample ID Chr Start Stop Length Value
6 NA16593 14 105587447 105849470 262023 1
4 NA16593 7 64316996 64819520 502524 1
5 NA16593 14 77977306 80668665 2691359 1
3 NA16593 5 7614901 22322881 14707980 1
2 NA16593 2 89665111 89885025 219914 1
1 NA16593 1 147305744 147467750 162006 1
0 NA16593 1 91902273 92863522 961249 3
7 NA16593 16 32349805 32762890 413085 1
8 NA16593 X 103148241 103174645 26404 3
9 NA16593 X 144917436 144981571 64135 1
Samples
Index Name ID Plate
1 Cri du Chat NA16593 WG0093128-DNA
2 Developmental Delay NA08585 WG0093128-DNA
3 Wolf-Hirschhorn Syndrome NA00343 WG0093128-DNA
4 Prader-Willi_rep NA12134_R WG0093128-DNA
5 Prader-Willi NA12134 WG0093128-DNA
6 Miller-Dieker Syndrome NA09208 WG0093128-DNA
7 Retinoblastoma NA04250 WG0093128-DNA
8 Turner Syndrome NA02668 WG0093128-DNA
Cri du Chat
B Allele Freq
DGV
Known Reg
Found Reg
5 Mb
Smoothed Log R
The project has been loaded successfully.
CHR 5 11291957:26000187

Figure 57 Panning Down to Examine the Boundaries of a Found Region

Notice that the red track indicating the found region has shifted higher on the screen.

NOTE

It is best to pan up until the start of the found region appears in the center of the chromosome browser--or pan down until the end of the found region appears in the center of the chromosome browser--so that when you zoom in further, the SNPs of interest are visible in the window.

Once your found region is centered in the chromosome browser, you can zoom in to get a higher-resolution view of the boundaries of your found region.

Alternatively, you can pan and zoom by selecting and dragging the red box which denotes the found region, as described in *Navigating the Chromosome Browser* on page 47.

3. Click **1/2X** **Zoom in 2x** or **1/5X** **Zoom in 5x** for a higher resolution view of your found region (Figure 58).

NOTE

If you want to view the boundaries of your found region at the SNP level, to see each individual data point in the plot, you may need to zoom in multiple times.

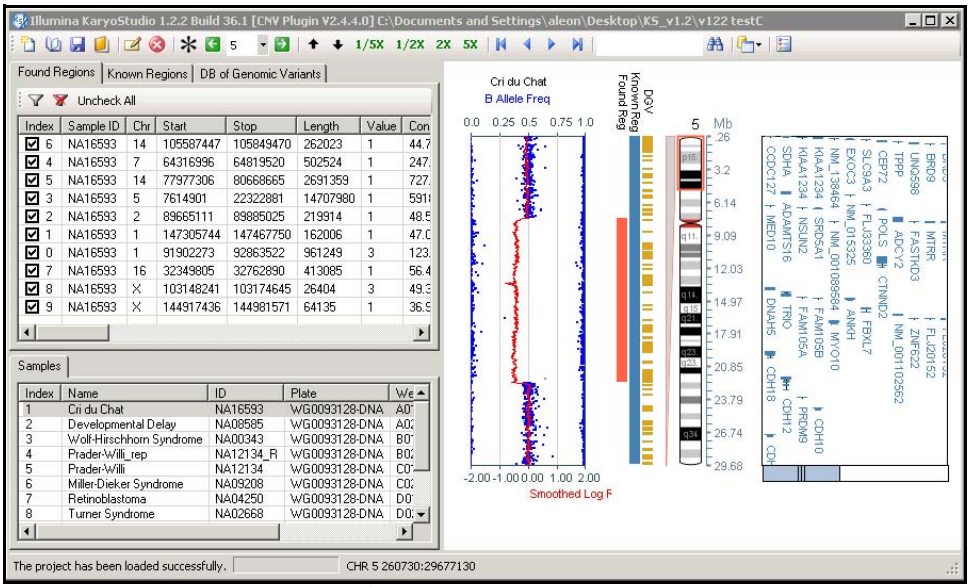


Figure 58 Centering a Found Region

4. To display a horizontal ruler, click anywhere on the ideogram. The horizontal ruler appears (Figure 59).

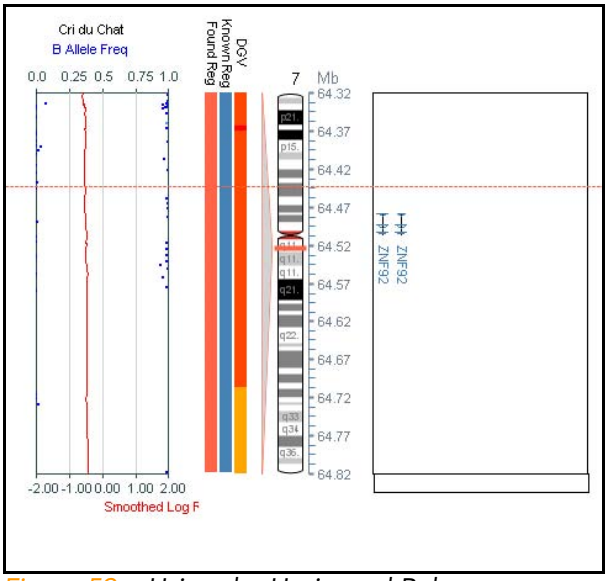


Figure 59 Using the Horizontal Ruler

5. Mouse over the ruler at any location to display a tooltip with the location coordinates (Figure 60).

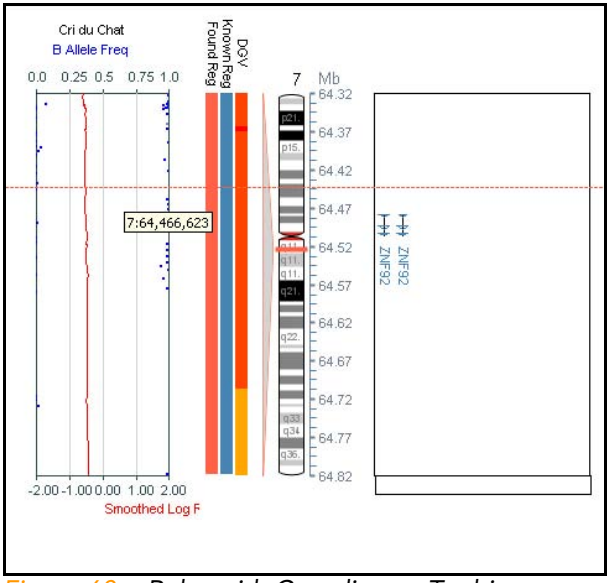


Figure 60 Ruler with Coordinates Tooltip

In most cases, the start and stop positions identified by the cnvPartition algorithm are very close to the positions you can identify by visual inspection. However, at this resolution, you have the option to edit the boundaries of the found region.

6. To edit the boundaries of a found region, right-click the found region of interest in the Found Regions table.

   A context menu appears (Figure 61).

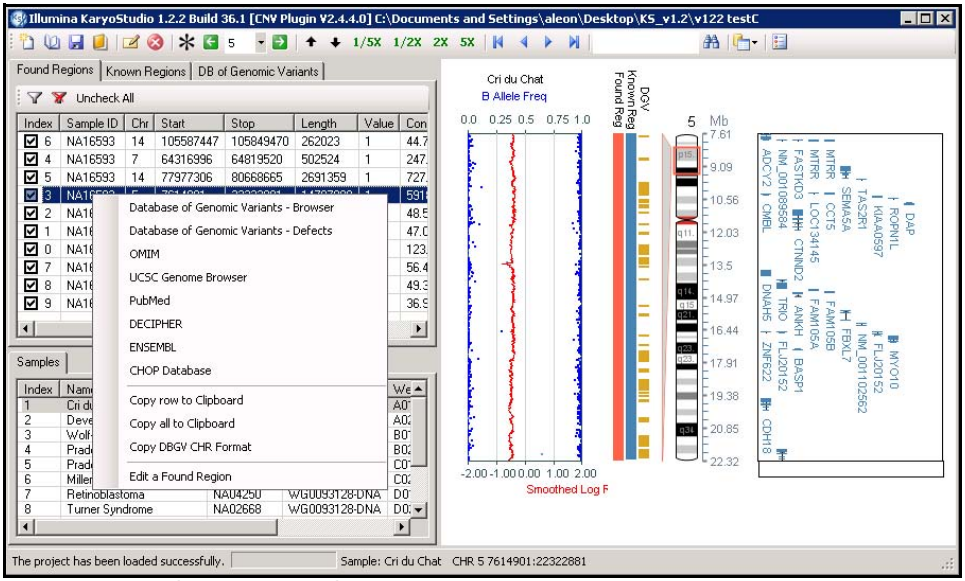


Figure 61 Editing a Found Region

7. Select **Edit a Found Region** from the context menu.

   The FormSettings screen appears (Figure 62).

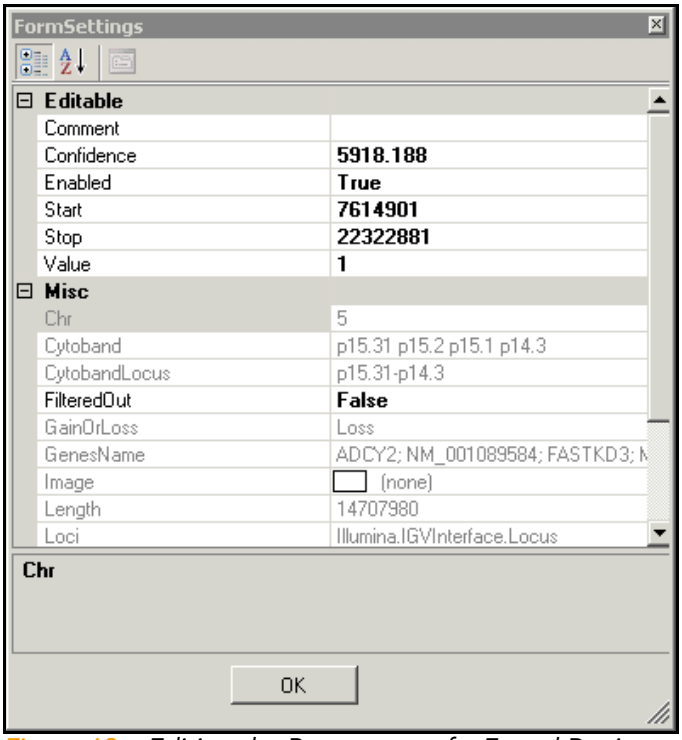


Figure 62 Editing the Parameters of a Found Region

NOTE

The FormSettings screen gives you the flexibility to adjust the Found Regions table a single row at a time. Repeat this process for each found region you want to edit.

8. Click **Start** or **Stop** and enter a new value to the right, based on the start or stop position you see in the chromosome browser.

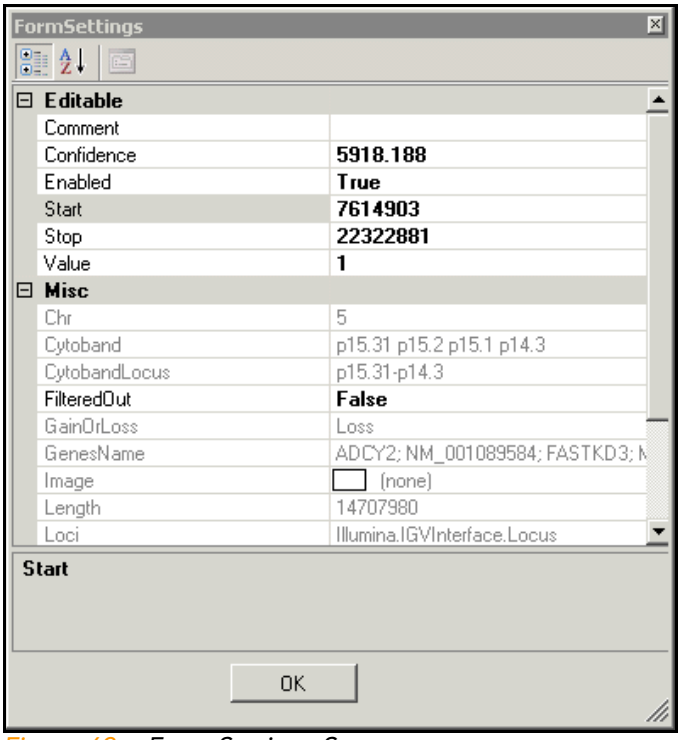


Figure 63 Form Settings Screen

9. Click **OK**.
10. The results of your change are not immediately visible in the chromosome browser. To see the change, click a different found region in the Found Regions table (Figure 63).

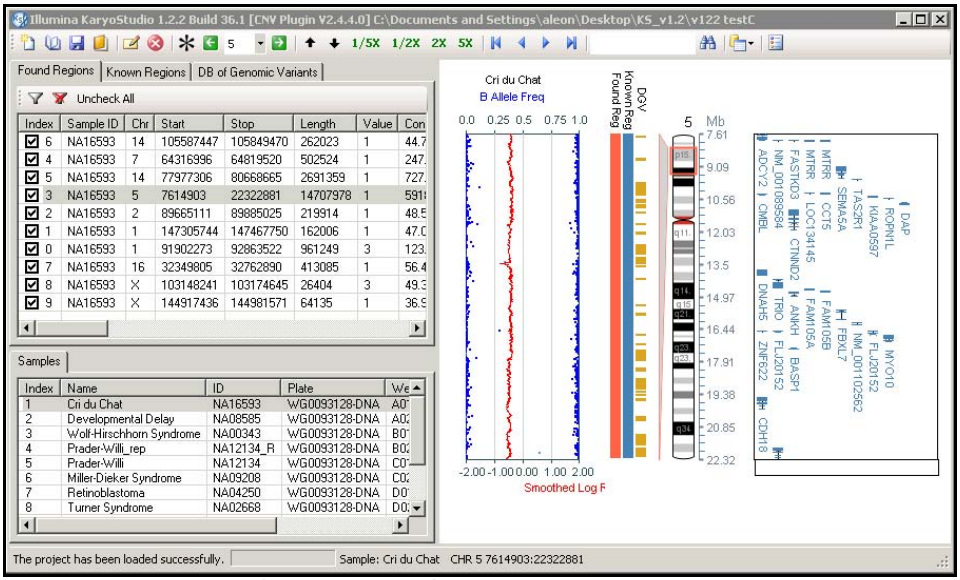

Illumina KaryoStudio 1.2.2 Build 36.1 [CNV Plugin V2.4.4.0] C:\Documents and Settings\aleon\Desktop\KS_v1.2\v122 testC
Found Regions
Known Regions
DB of Genomic Variants
Uncheck All
Index Sample ID Chr Start Stop Length Value
6 NA16593 14 105587447 105849470 262023 1
4 NA16593 7 64316996 64819520 502524 1
5 NA16593 14 77977306 80668665 2691359 1
3 NA16593 5 7614903 22322881 14707978 1
2 NA16593 2 89665111 89885025 219914 1
1 NA16593 1 147305744 147467750 162006 1
0 NA16593 1 91902273 92863522 961249 3
7 NA16593 16 32349805 32762890 413085 1
8 NA16593 X 103148241 103174645 26404 3
9 NA16593 X 144917436 144981571 64135 1
Samples
Index Name ID Plate
1 Cri du Chat NA16593 WG0093128-DNA
2 Developmental Delay NA08585 WG0093128-DNA
3 Wolf-Hirschhorn Syndrome NA00343 WG0093128-DNA
4 Prader-Willi_rep NA12134_R WG0093128-DNA
5 Prader-Willi NA12134 WG0093128-DNA
6 Miller-Dieker Syndrome NA09208 WG0093128-DNA
7 Retinoblastoma NA04250 WG0093128-DNA
8 Turner Syndrome NA02668 WG0093128-DNA
Cri du Chat
B Allele Freq
Smoothed Log R
The project has been loaded successfully.
Sample: Cri du Chat
CHR 5 7614903:22322881

Figure 64 Viewing the New Found Region Positions

The new start and stop regions are included in the Found Regions table, in the Found Regions track, and in all subsequent analyses.

Exporting Found Regions

If you want to export data from the Found Regions table, you can export a single row of data, or the entire table.

- To export a single row:

  Right click in the Found Regions table and select **Copy Row to Clipboard**.

- To export the whole table:

  Right-click in the Found Regions table and select **Copy All to Clipboard** (Figure 65).

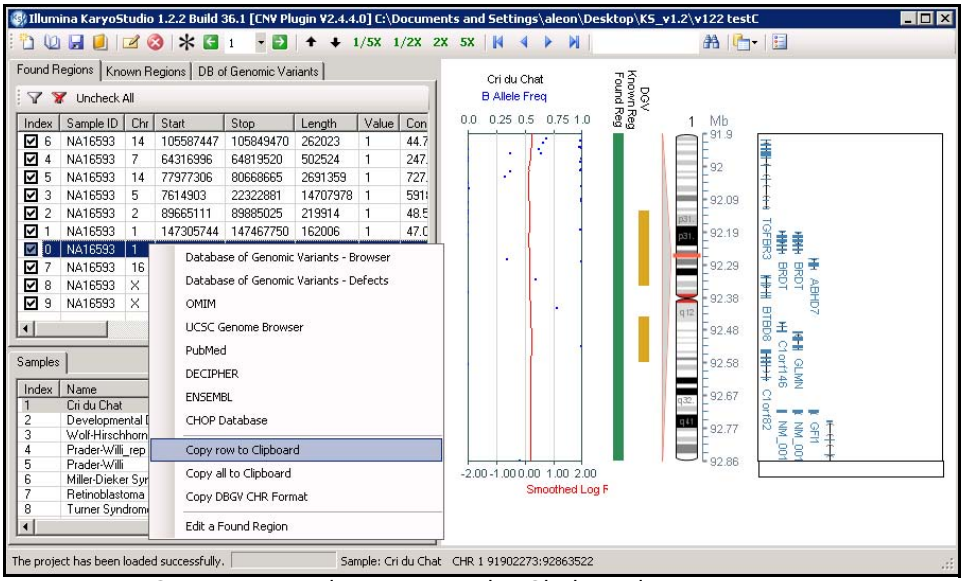


Figure 65 Copying Found Regions to the Clipboard

You can now paste this data into an Excel file or import it into other downstream, third-party applications.

The checkboxes to the left of the Index numbers indicate whether or not a found region will be included in your report.

All checkboxes are selected by default, which means that all found regions will be included in your report by default.

To deselect all regions, click **Uncheck All**. To select all regions, click **Check All**.

To exclude certain found regions from your report, deselect the checkbox(es) to the left of the region(s) you want to exclude.

Chapter 5

Cross-Matching to Known Variants

Topics

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Introduction

Because a sample may potentially contain many aberrations, it is especially important to know what has already been identified as a normal variation or a region associated with a condition. For this reason, KaryoStudio allows you to check a found region against a list of known regions as well as several different external sources. This function allows you to determine which regions found in your samples may be the most relevant.

This chapter describes how to link to these external information sources from within KaryoStudio, and how to use this additional information in your study.

KaryoStudio allows you to link to several databases, including:

- **DGV (Database of Genomic Variants)**—to determine whether your region has been identified as a copy number variant in normal, healthy individuals
- **OMIM (On-line Mendelian Inheritance of Man)**—to allow further mining of a particular phenotype, region, or gene of interest
- **UCSC Genome Browser**—to view genes or numerous other information tracks for a particular region in this popular public genome browser
- **PubMed**—to research publications relevant to a particular phenotype/gene of interest
- **DECIPHER**—to compare found regions to the DECIPHER database of submicroscopic chromosomal imbalances
- **Ensembl**—to view a found region in the graphical Ensembl genome viewer
- **CHOP Database**—to determine whether a found region overlaps with copy number variations described in this database of samples from healthy individuals

Database of Genomic Variants (DGV)

Since the widespread adoption of high-density arrays for screening the genome for structural changes, many studies have identified various segments of the genome of differing sizes that range in copy number. From arrays to sequencing, the number of regions is growing dramatically and includes both common (>5%) and rare (1%) regions. Although many of these regions are not yet linked to specific conditions, the hope is that these studies will have a major impact on human health.

The DGV is one of the databases that is collating these regions in the hope of providing a comprehensive summary of structural variation human genomes. This database includes regions of the genome larger than >1kb that were identified in various studies of healthy control samples.

Updating KaryoStudio with the Latest DGV Build

The DGV currently includes many known CNV regions and covers the majority of the genome.

To update KaryoStudio with the latest DGV build, perform the following steps:

1. Find the latest version of the Genomic Variation Table at the Database of Genomic Variants web site:

   http://projects.tcag.ca/variation/

   and click the Downloads section.

2. Save the latest version of the Genomic Variation Table as a file of name *GenomicVariation.txt* in the following location:

   C:\Program Files\Illumina KaryoStudio

3. Restart KaryoStudio to load the updated table.

Using the DGV with KaryoStudio

You can use KaryoStudio to see if a region identified by KaryoStudio has already been identified and deposited in the DGV. Based upon chromosome and position, the DGV shows you information about what is known about the method used to identify the region, the reference (including details of the study), and a graphical reference to any CNVs found in the region.

Perform the following steps to use the DGV with KaryoStudio:

1. In the Found Regions table, select the region you want to search for in the DGV (Figure 66).

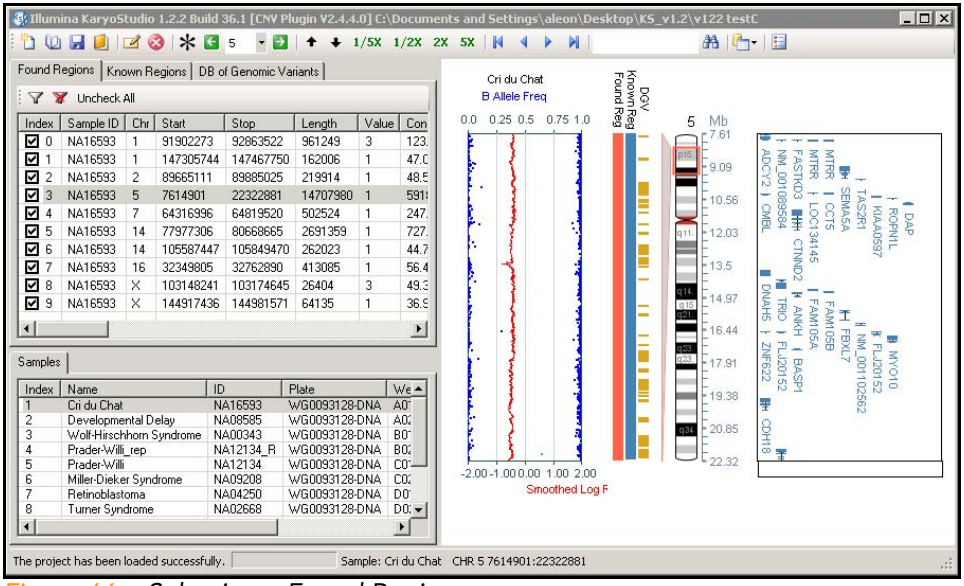

Illumina KaryoStudio 1.2.2 Build 36.1 [CNV Plugin V2.4.4.0] C:\Documents and Settings\aleon\Desktop\KS_v1.2\v122 testC
Found Regions
Known Regions
DB of Genomic Variants
Uncheck All
Index Sample ID Chr Start Stop Length Value
0 NA16593 1 91902273 92863522 961249 3
1 NA16593 1 147305744 147467750 162006 1
2 NA16593 2 89665111 89885025 219914 1
3 NA16593 5 7614901 22322881 14707980 1
4 NA16593 7 64316996 64819520 502524 1
5 NA16593 14 77977306 80668665 2691359 1
6 NA16593 14 105587447 105849470 262023 1
7 NA16593 16 32349805 32762890 413085 1
8 NA16593 X 103148241 103174645 26404 3
9 NA16593 X 144917436 144981571 64135 1
Samples
Index Name ID Plate
1 Cri du Chat NA16593 WG0093128-DNA
2 Developmental Delay NA08585 WG0093128-DNA
3 Wolf-Hirschhorn Syndrome NA00343 WG0093128-DNA
4 Prader-Willi_rep NA12134_R WG0093128-DNA
5 Prader-Willi NA12134 WG0093128-DNA
6 Miller-Dieker Syndrome NA09208 WG0093128-DNA
7 Retinoblastoma NA04250 WG0093128-DNA
8 Turner Syndrome NA02668 WG0093128-DNA
Cri du Chat
B Allele Freq
Known Reg
Found Reg
DGV
Mb
Smoothed Log R
The project has been loaded successfully.
Sample: Cri du Chat CHR 5 7614901:22322881

Figure 66 Selecting a Found Region

2. Right-click the region, and select **Database of Genomic Variants - Browser** from the context menu (Figure 67).

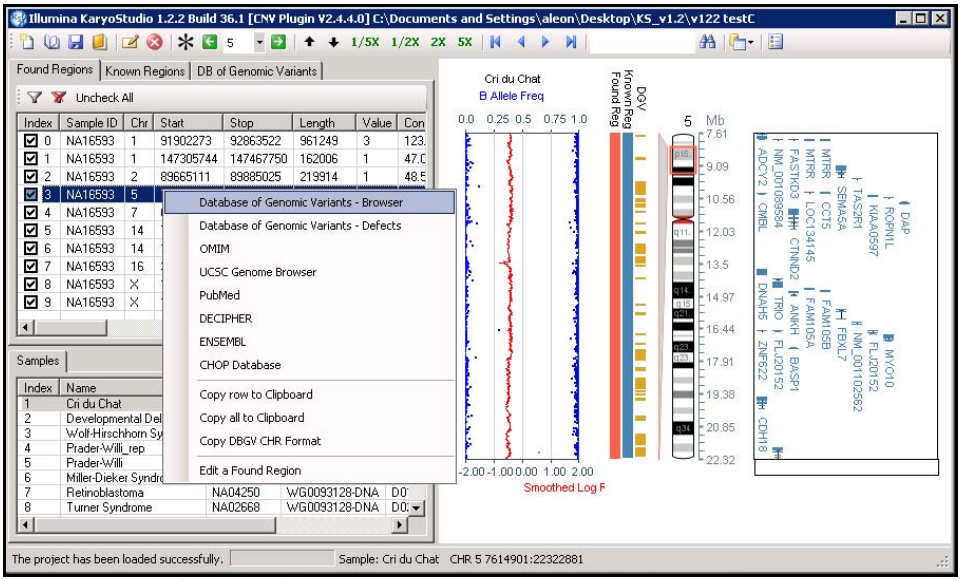


Figure 67 Selecting the DGV

KaryoStudio sends the chromosome start and stop position information of the found region to the DGV, which notifies you of how many matches were found, and displays a genome browser view of that region (Figure 68).

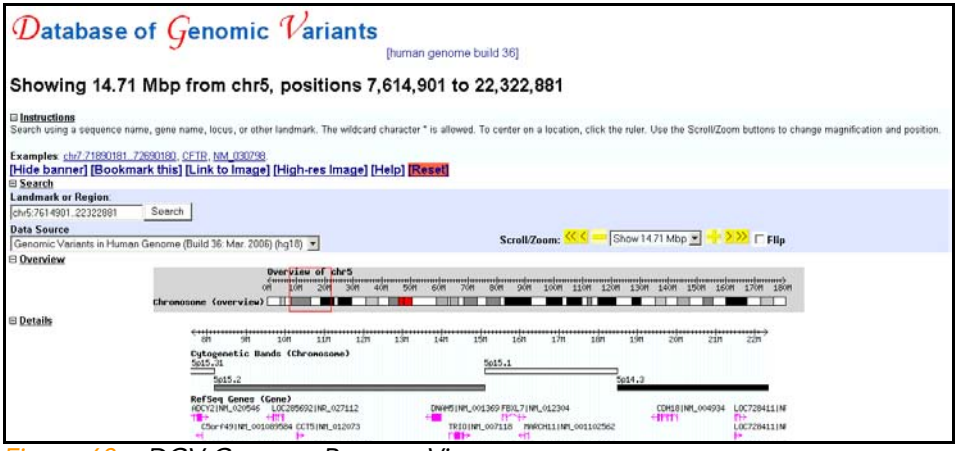


Figure 68 DGV Genome Browser View

NOTE

Many optional tracks can be displayed in the DGV browser view. Refer to the documentation supplied with the Database of Genomic Variants for more information about using and interpreting information in the browser.

3. Scroll down until you get to the section labeled **All CNVs** (Figure 69).

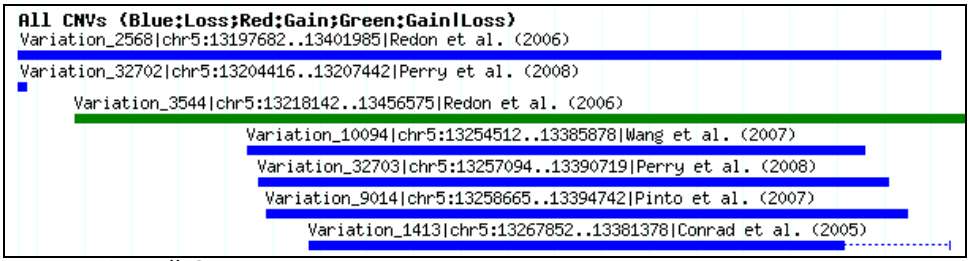


Figure 69 All CNVs

The All CNVs section shows colored bars representing all CNVs present in the database for the genomic region in the view.

4. Inspect a variation entry by clicking it.

   A new window opens (Figure 70) with details about the selected entry, including:

   - variation number
   - cytogenetic band
   - genes
   - other overlapping CNVs
   - genomic coordinates
   - nearby segmental duplications
   - study details
   - references to the study that reported the variant

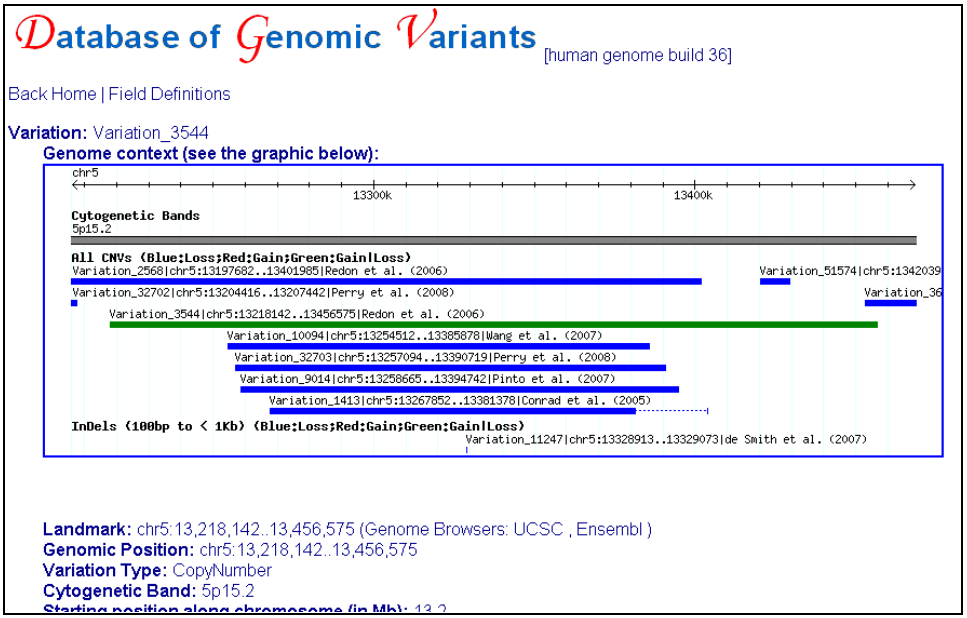


Figure 70 Inspecting a Variation

For the purposes of this example, Variation_3544, shown above, is used.

Notice the variation number, Variation_3544, in the upper-left of the screen (Figure 71).

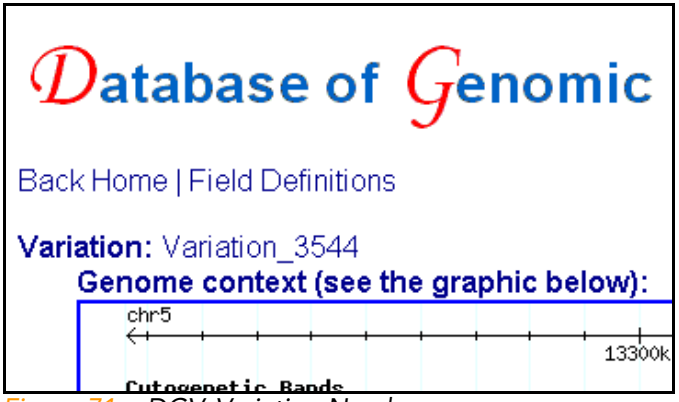


Figure 71 DGV, Variation Number

This variation number is a permanent number assigned to the region. If your lab is interested in this region, you can use this ID to refer to this region in the DGV.

Next, notice the cytogenetic band (Figure 72).

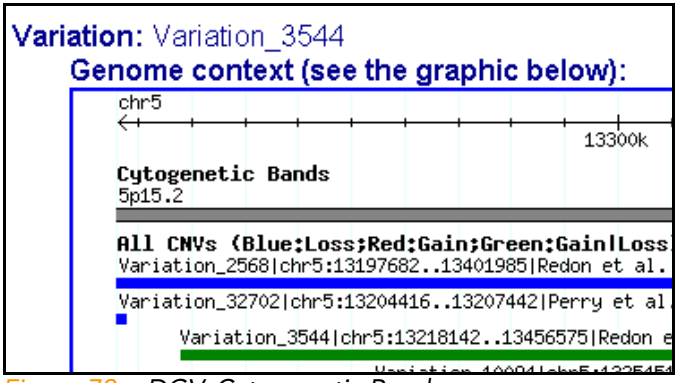


Figure 72 DGV, Cytogenetic Band

This tells you which cytoband your region sits in. This is particularly important if you already know that a certain condition is associated with a change in a specific cytoband.

The next section, All CNVs, tells you whether any other CNVs have been identified in this region (Figure 73).

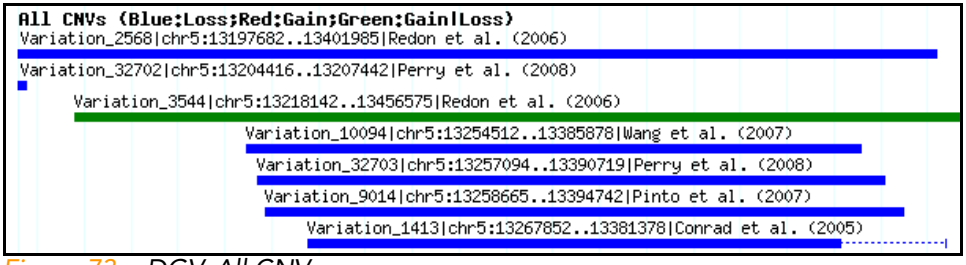


Figure 73 DGV, All CNVs

In addition, start and stop positions of each region, and the study in which the region was identified are shown. You can click a variation for more information.

The sections below the CNVs contain information about genomic inversions, indels, segmental duplications, etc. In Figure 74, only indels are shown.

Figure 74 DGV, Indels

You can click each available section for more information that you may want to incorporate into your cytogenetics study.

Below the graphic, there is some additional information (Figure 75).

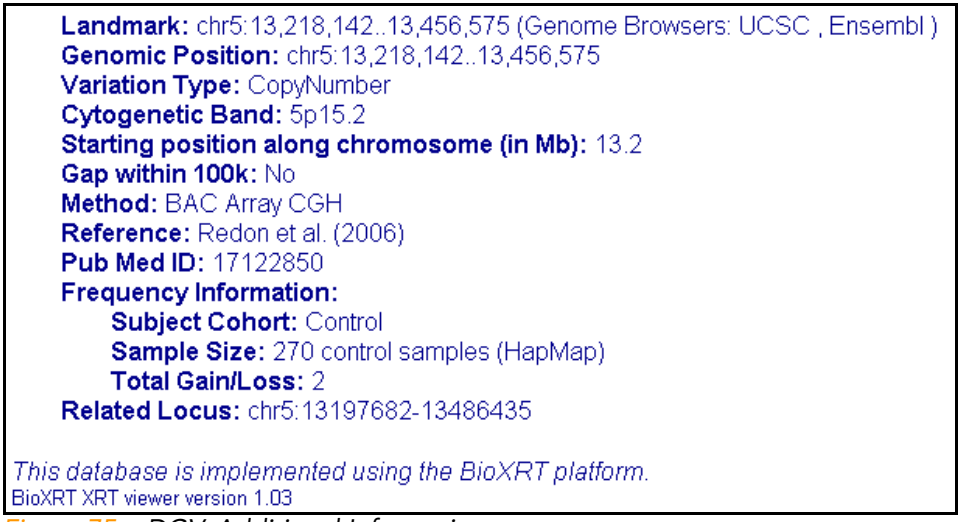

Landmark: chr5:13,218,142..13,456,575 (Genome Browsers: UCSC , Ensembl)
Genomic Position: chr5:13,218,142..13,456,575
Variation Type: CopyNumber
Cytogenetic Band: 5p15.2
Starting position along chromosome (in Mb): 13.2
Gap within 100k: No
Method: BAC Array CGH
Reference: Redon et al. (2006)
Pub Med ID: 17122850
Frequency Information:
Subject Cohort: Control
Sample Size: 270 control samples (HapMap)
Total Gain/Loss: 2
Related Locus: chr5:13197682-13486435
This database is implemented using the BioXRT platform.
BioXRT XRT viewer version 1.03

Figure 75 DGV, Additional Information

The frequency information may be particularly useful for interpretation. It includes the number of samples studied, and the number of times a loss or gain was seen.

Notice that you can click a link to PubMed (if available), which displays the publication reference for the study that identified this region (Figure 76).

NCBI Resources How To
My NCBI | Sign In
PubMed.gov
U.S. National Library of Medicine
National Institutes of Health
Search: PubMed
Limits Advanced search Help
Search Clear
Display Settings: Abstract
Send to:
nature
Related articles
Nature. 2006 Nov 23;444(7118):444-54.
Global variation in copy number in the human genome.
Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, González JR, Gratacós M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME.
The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK.
Comment in:
Nature. 2006 Nov 23;444(7118):428-9.
Copy number variation (CNV) of DNA sequences is functionally significant but has yet to be fully ascertained. We have constructed a first-generation CNV map of the human genome through the study of 270 individuals from four populations with ancestry in Europe, Africa or Asia (the HapMap collection). DNA from these individuals was screened for CNV using two complementary technologies: single-nucleotide polymorphism (SNP) genotyping arrays, and clone-based comparative genomic hybridization. A total of 1,447 copy number variable regions (CNVRs), which can encompass overlapping or adjacent gains or losses, covering 360 megabases (12% of the genome) were identified in these populations. These CNVRs contained hundreds of genes, disease loci, functional elements and segmental duplications. Notably, the CNVRs encompassed more nucleotide content per genome than SNPs, underscoring the importance of CNV in genetic diversity and evolution. The data obtained delineate linkage disequilibrium patterns for many CNVs, and reveal marked variation in copy number among populations. We also demonstrate the utility of this resource for genetic disease studies.
PMID: 17122850 [PubMed - indexed for MEDLINE]
PMCID: PMC2669898
Publication Types, MeSH Terms, Grant Support
LinkOut - more resources
Cited by over 100 PubMed Central articles

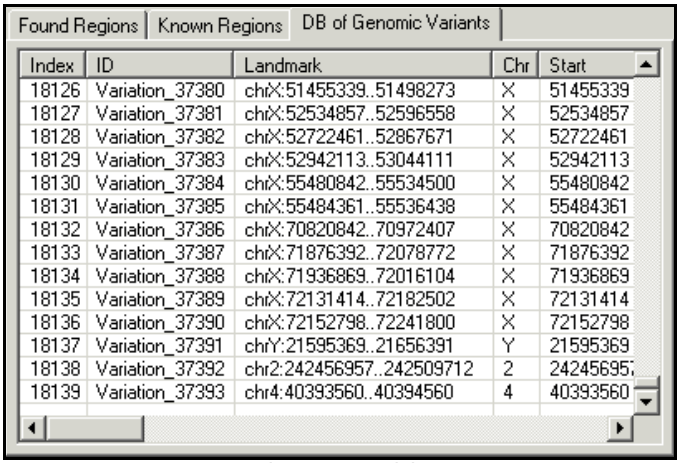
Figure 76 DGV, PubMed Reference

The DGV is a resource provided by SickKids Hospital in Toronto, Canada. If you need more information about this database, go to http://projects/tcag.ca/, or send email to dgv-contact@sickkids.ca.

All of the CNVs in the DGV were identified in healthy control samples. The DGV is constantly being updated as new studies deposit information and as technology evolves. For the latest build, and latest information, please see the DGV web page: http://projects.tcag.ca/variation/.

DGV Data

When you click the DGV tab, it may take a moment to load. When it does, scroll to the bottom of the list (Figure 77).



Index	ID	Landmark	Chr	Start
18126	Variation_37380	chrX:51455339..51498273	X	51455339
18127	Variation_37381	chrX:52534857..52596558	X	52534857
18128	Variation_37382	chrX:52722461..52867671	X	52722461
18129	Variation_37383	chrX:52942113..53044111	X	52942113
18130	Variation_37384	chrX:55480842..55534500	X	55480842
18131	Variation_37385	chrX:55484361..55536438	X	55484361
18132	Variation_37386	chrX:70820842..70972407	X	70820842
18133	Variation_37387	chrX:71876392..72078772	X	71876392
18134	Variation_37388	chrX:71936869..72016104	X	71936869
18135	Variation_37389	chrX:72131414..72182502	X	72131414
18136	Variation_37390	chrX:72152798..72241800	X	72152798
18137	Variation_37391	chrY:21595369..21656391	Y	21595369
18138	Variation_37392	chr2:242456957..242509712	2	24245695
18139	Variation_37393	chr4:40393560..40394560	4	40393560

Figure 77 KaryoStudio DGV Table

If you want to see whether any of the found regions in your data match known DGV regions, click a region in the DGV table. The data for the region you clicked appears in the chromosome browser (Figure 78).

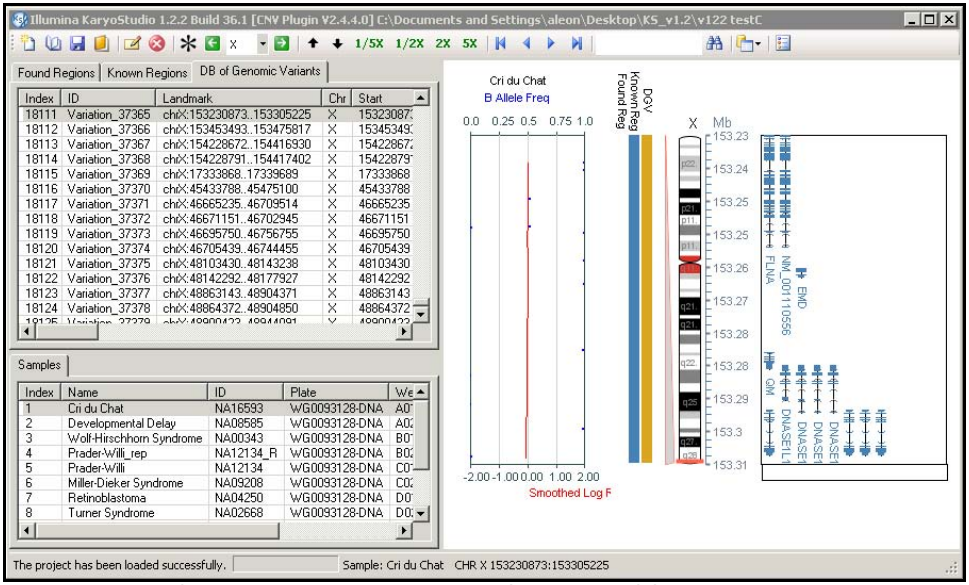

Illumina KaryoStudio 1.2.2 Build 36.1 [CNV Plugin V2.4.4.0] C:\Documents and Settings\aleon\Desktop\KS_v1.2\v122 testC
Found Regions
Known Regions
DB of Genomic Variants
Samples
Cri du Chat
B Allele Freq
Smoothed Log R
The project has been loaded successfully.
Sample: Cri du Chat CHR X 153230873:153305225

Figure 78 Selecting a Known Region in the DGV Table

You can use this option if you prefer to view CNV data within KaryoStudio rather than linking out to the DGV.

The advantage of viewing your data in KaryoStudio is that you can look at a CNV region across multiple samples.

Updating the DGV Table and Track

To update the data used to populate the DGV table and track in KaryoStudio, perform the following steps:

1. Download the database build from the following location: http://projects/tcag.ca/variation/tableview.asp?table=DGV_Content_Summary.txt.
2. Download the variation file (*.txt format) in the Current Version section, based on Build 36 (hg 18) of the genome, to your computer.

   Example variation file: *variation.hg18.v8.aug.2009.txt*
3. Rename this file "*DatabaseOfGenomicVariation.txt*".
4. Copy the file *DatabaseOfGenomicVariation.txt* (replacing the existing file) to the directory *C:\Program Files\Illumina\Illumina KaryoStudio*.
5. Restart KaryoStudio to load the new data file.

OMIM (Online Mendelian Inheritance of Man)

The OMIM database, hosted by the National Center for Biotechnology Information (NCBI), contains information on human genes and genetic phenotypes on all known Mendelian disorders and over 12,000 genes. OMIM focuses on the relationship between phenotype and genotype.

Using OMIM with KaryoStudio

Because OMIM is based upon specific keywords and not basic chromosome start and stop positions, KaryoStudio only provides a link to the homepage of this database. If KaryoStudio has identified a specific phenotype within the found regions table, you can enter a key word (e.g., gene, cytoband, or phenotype) in the homepage of OMIM to obtain more information if available.

To use OMIM with KaryoStudio, perform the following steps:

1. Select a region of interest in the Found Regions table.
2. Right-click and select **OMIM** from the context menu (Figure 79).

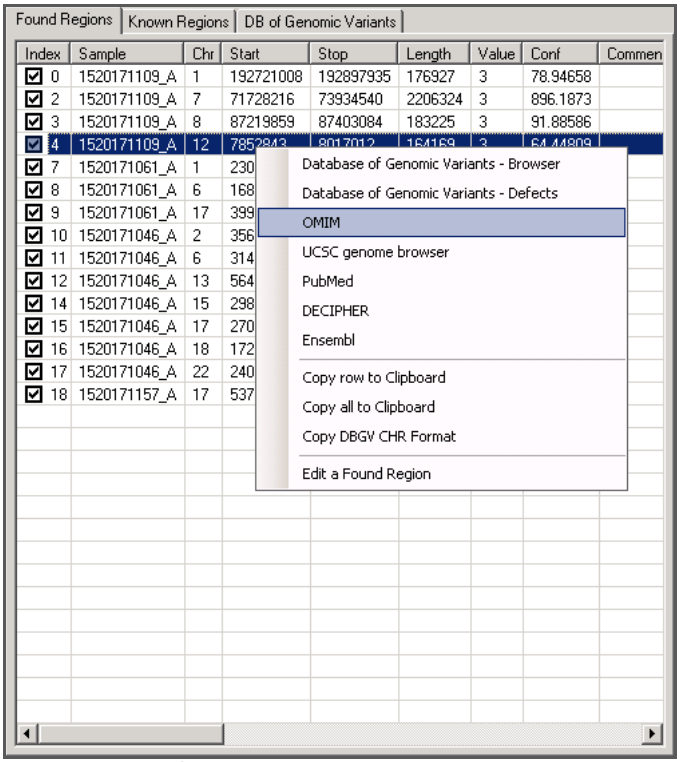


Figure 79 Selecting OMM

OMIM opens to the homepage (Figure 80).

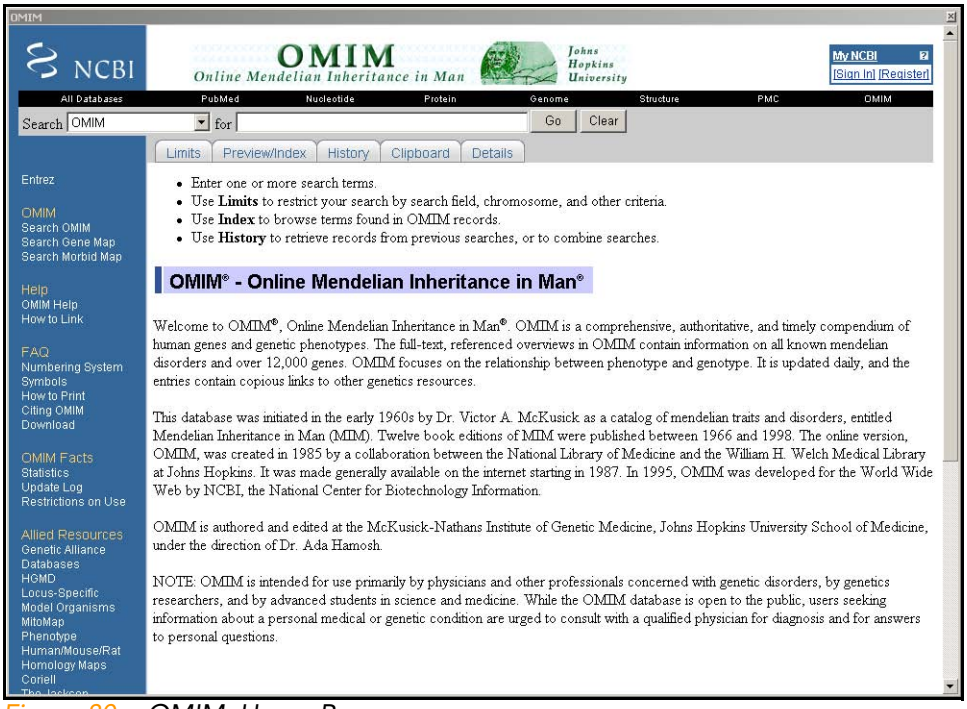

OMIM
NCBI
OMIM
Online Mendelian Inheritance in Man
Johns Hopkins University
My NCBI
[Sign In] [Register]
All Databases PubMed Nucleotide Protein Genome Structure PMC OMIM
Search OMIM for
Go Clear
Limits Preview/Index History Clipboard Details
Entrez
OMIM
Search OMIM
Search Gene Map
Search Morbid Map
Help
OMIM Help
How to Link
FAQ
Numbering System
Symbols
How to Print
Citing OMIM
Download
OMIM Facts
Statistics
Update Log
Restrictions on Use
Allied Resources
Genetic Alliance
Databases
HGMD
Locus-Specific
Model Organisms
MitoMap
Phenotype
Human/Mouse/Rat
Homology Maps
Coriell
• Enter one or more search terms.
• Use Limits to restrict your search by search field, chromosome, and other criteria.
• Use Index to browse terms found in OMIM records.
• Use History to retrieve records from previous searches, or to combine searches.
OMIM® - Online Mendelian Inheritance in Man®
Welcome to OMIM®, Online Mendelian Inheritance in Man®. OMIM is a comprehensive, authoritative, and timely compendium of human genes and genetic phenotypes. The full-text, referenced overviews in OMIM contain information on all known mendelian disorders and over 12,000 genes. OMIM focuses on the relationship between phenotype and genotype. It is updated daily, and the entries contain copious links to other genetics resources.
This database was initiated in the early 1960s by Dr. Victor A. McKusick as a catalog of mendelian traits and disorders, entitled Mendelian Inheritance in Man (MIM). Twelve book editions of MIM were published between 1966 and 1998. The online version, OMIM, was created in 1985 by a collaboration between the National Library of Medicine and the William H. Welch Medical Library at Johns Hopkins. It was made generally available on the internet starting in 1987. In 1995, OMIM was developed for the World Wide Web by NCBI, the National Center for Biotechnology Information.
OMIM is authored and edited at the McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, under the direction of Dr. Ada Hamosh.
NOTE: OMIM is intended for use primarily by physicians and other professionals concerned with genetic disorders, by genetics researchers, and by advanced students in science and medicine. While the OMIM database is open to the public, users seeking information about a personal medical or genetic condition are urged to consult with a qualified physician for diagnosis and for answers to personal questions.

Figure 80 OMIM, Home Page

3. Type a key word in the search text field.

   Key words can be gene names, etc. For example, if you had identified the MYC gene in your search, you might want to enter MYC as a key word. OMIM identifies your gene in multiple organisms and displays a list of the occurrences of this gene in all organisms (Figure 81).

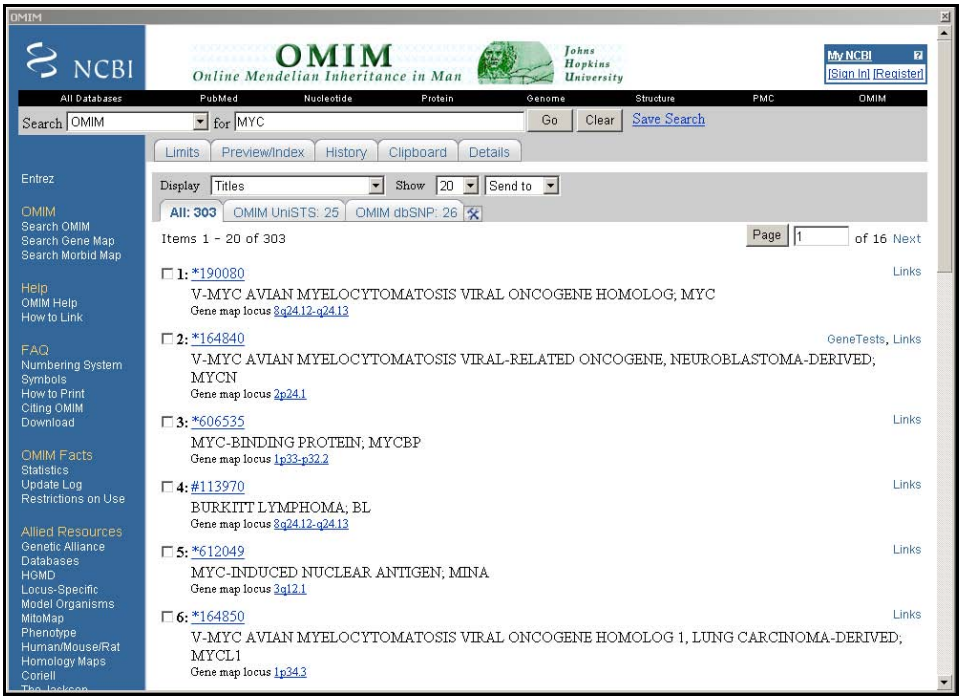


Figure 81 OMIM, MYC Gene

If you want to look at this gene in the human genome only, for example, you must scroll down to find it in the list.

If you click on a hit, information about this gene is displayed, including the name, description, etc.

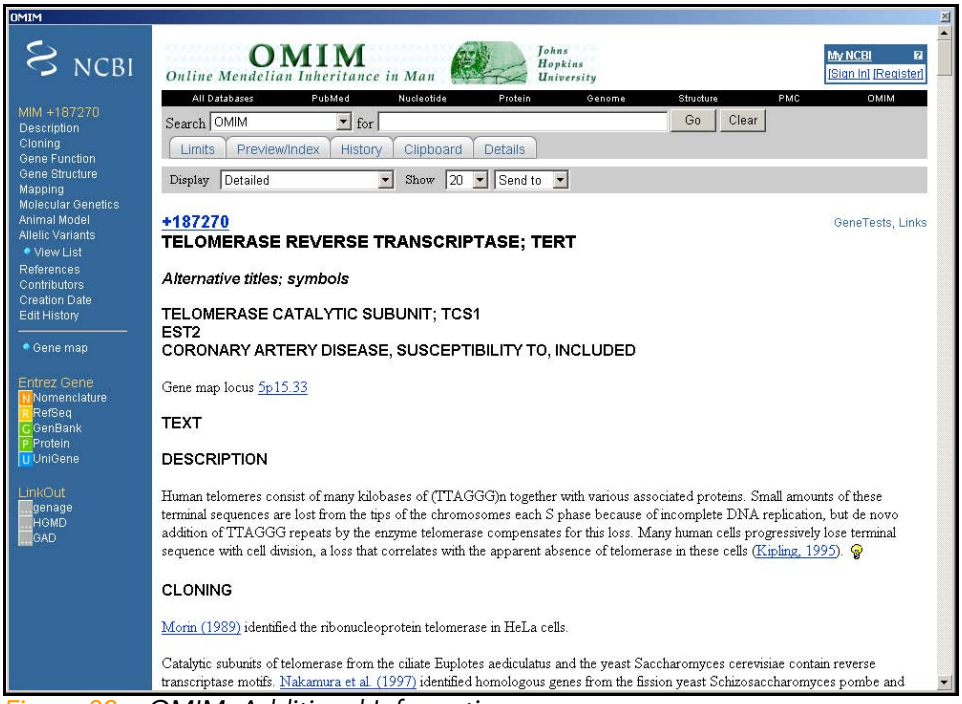

OMIM
NCBI
OMIM
Online Mendelian Inheritance in Man
Johns Hopkins University
My NCBI
[Sign In] [Register]
All Databases PubMed Nucleotide Protein Genome Structure PMC OMIM
Search OMIM for Go Clear
Limits Preview/Index History Clipboard Details
Display Detailed Show 20 Send to
MIM +187270
Description
Cloning
Gene Function
Gene Structure
Mapping
Molecular Genetics
Animal Model
Allelic Variants
View List
References
Contributors
Creation Date
Edit History
Gene map
Entrez Gene
Nomenclature
RefSeq
GenBank
Protein
UniGene
LinkOut
genage
HGMD
GAD
+187270
GeneTests, Links
TELOMERASE REVERSE TRANSCRIPTASE; TERT
Alternative titles; symbols
TELOMERASE CATALYTIC SUBUNIT; TCS1
EST2
CORONARY ARTERY DISEASE, SUSCEPTIBILITY TO, INCLUDED
Gene map locus 5p15.33
TEXT
DESCRIPTION
Human telomeres consist of many kilobases of (TTAGGG)n together with various associated proteins. Small amounts of these terminal sequences are lost from the tips of the chromosomes each S phase because of incomplete DNA replication, but de novo addition of TTAGGG repeats by the enzyme telomerase compensates for this loss. Many human cells progressively lose terminal sequence with cell division, a loss that correlates with the apparent absence of telomerase in these cells (Kipling, 1995).
CLONING
Morin (1989) identified the ribonucleoprotein telomerase in HeLa cells.
Catalytic subunits of telomerase from the ciliate Euplotes aediculatus and the yeast Saccharomyces cerevisiae contain reverse transcriptase motifs. Nakamura et al. (1997) identified homologous genes from the fission yeast Schizosaccharomyces pombe and

Figure 82 OMIM, Additional Information

Of particular interest is the Gene Function section, which describes any known molecular function of this gene (Figure 83).

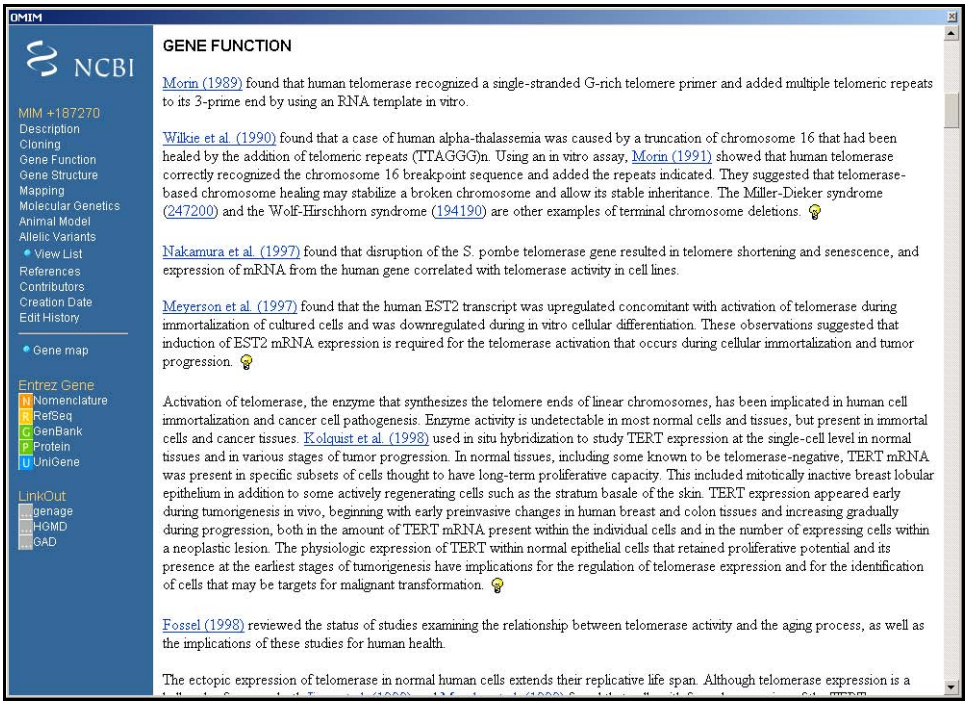

OMIM
NCBI
MIM #187270
Description
Cloning
Gene Function
Gene Structure
Mapping
Molecular Genetics
Animal Model
Allelic Variants
View List
References
Contributors
Creation Date
Edit History
Gene map
Entrez Gene
Nomenclature
RefSeq
GenBank
Protein
UniGene
LinkOut
genage
HGMD
GAD
GENE FUNCTION
Morin (1989) found that human telomerase recognized a single-stranded G-rich telomere primer and added multiple telomeric repeats to its 3-prime end by using an RNA template in vitro.
Wilkie et al. (1990) found that a case of human alpha-thalassemia was caused by a truncation of chromosome 16 that had been healed by the addition of telomeric repeats (TTAGGG)n. Using an in vitro assay, Morin (1991) showed that human telomerase correctly recognized the chromosome 16 breakpoint sequence and added the repeats indicated. They suggested that telomerase-based chromosome healing may stabilize a broken chromosome and allow its stable inheritance. The Miller-Dieker syndrome (247200) and the Wolf-Hirschhorn syndrome (194190) are other examples of terminal chromosome deletions.
Nakamura et al. (1997) found that disruption of the S. pombe telomerase gene resulted in telomere shortening and senescence, and expression of mRNA from the human gene correlated with telomerase activity in cell lines.
Meyerson et al. (1997) found that the human EST2 transcript was upregulated concomitant with activation of telomerase during immortalization of cultured cells and was downregulated during in vitro cellular differentiation. These observations suggested that induction of EST2 mRNA expression is required for the telomerase activation that occurs during cellular immortalization and tumor progression.
Activation of telomerase, the enzyme that synthesizes the telomere ends of linear chromosomes, has been implicated in human cell immortalization and cancer cell pathogenesis. Enzyme activity is undetectable in most normal cells and tissues, but present in immortal cells and cancer tissues. Kolquist et al. (1998) used in situ hybridization to study TERT expression at the single-cell level in normal tissues and in various stages of tumor progression. In normal tissues, including some known to be telomerase-negative, TERT mRNA was present in specific subsets of cells thought to have long-term proliferative capacity. This included mitotically inactive breast lobular epithelium in addition to some actively regenerating cells such as the stratum basale of the skin. TERT expression appeared early during tumorigenesis in vivo, beginning with early preinvasive changes in human breast and colon tissues and increasing gradually during progression, both in the amount of TERT mRNA present within the individual cells and in the number of expressing cells within a neoplastic lesion. The physiologic expression of TERT within normal epithelial cells that retained proliferative potential and its presence at the earliest stages of tumorigenesis have implications for the regulation of telomerase expression and for the identification of cells that may be targets for malignant transformation.
Fossel (1998) reviewed the status of studies examining the relationship between telomerase activity and the aging process, as well as the implications of these studies for human health.
The ectopic expression of telomerase in normal human cells extends their replicative life span. Although telomerase expression is a

Figure 83 OMIM, Gene Function

If you enter a key word that is a condition in the OMIM search field, OMIM provides information about that condition.

For example, enter "down syndrome." A listing page comes up with the closest matches.

Click the first entry, **#190685 DOWN SYNDROME**. A down syndrome information page appears (Figure 84).

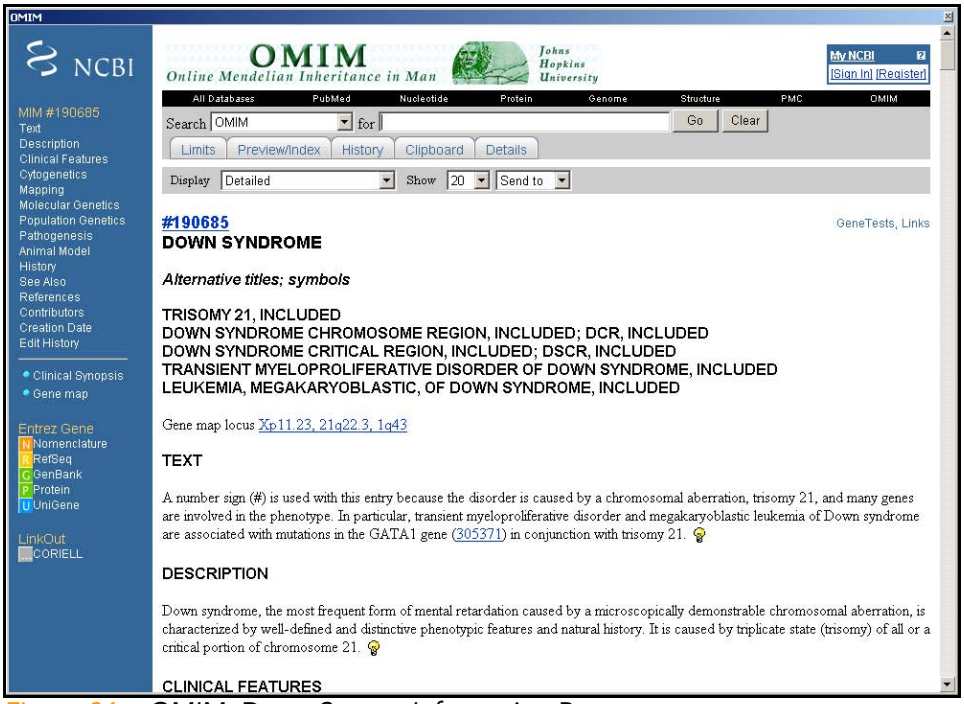

OMIM
NCBI
OMIM
Online Mendelian Inheritance in Man
Johns Hopkins University
My NCBI
[Sign In] [Register]
All Databases PubMed Nucleotide Protein Genome Structure PMC OMIM
Search OMIM for Go Clear
Limits Preview/Index History Clipboard Details
Display Detailed Show 20 Send to
MIM #190685
Text
Description
Clinical Features
Cytogenetics
Mapping
Molecular Genetics
Population Genetics
Pathogenesis
Animal Model
History
See Also
References
Contributors
Creation Date
Edit History
Clinical Synopsis
Gene map
Entrez Gene
Nomenclature
RefSeq
GenBank
Protein
UniGene
LinkOut
CORIELL
#190685
DOWN SYNDROME
GeneTests, Links
Alternative titles; symbols
TRISOMY 21, INCLUDED
DOWN SYNDROME CHROMOSOME REGION, INCLUDED; DCR, INCLUDED
DOWN SYNDROME CRITICAL REGION, INCLUDED; DSCR, INCLUDED
TRANSIENT MYELOPROLIFERATIVE DISORDER OF DOWN SYNDROME, INCLUDED
LEUKEMIA, MEGAKARYOBLASTIC, OF DOWN SYNDROME, INCLUDED
Gene map locus Xp11.23, 21q22.3, 1q43
TEXT
A number sign (#) is used with this entry because the disorder is caused by a chromosomal aberration, trisomy 21, and many genes are involved in the phenotype. In particular, transient myeloproliferative disorder and megakaryoblastic leukemia of Down syndrome are associated with mutations in the GATA1 gene (305371) in conjunction with trisomy 21.
DESCRIPTION
Down syndrome, the most frequent form of mental retardation caused by a microscopically demonstrable chromosomal aberration, is characterized by well-defined and distinctive phenotypic features and natural history. It is caused by triplicate state (trisomy) of all or a critical portion of chromosome 21.
CLINICAL FEATURES

Figure 84 OMIM, Down System Information Page

Information such as a description of the condition, clinical features, and more is provided.

UCSC Genome Browser

The UCSC Genome Browser is a popular tool that allows you to examine gene information, expression data, and many other parameters within your found region. Yo can also upload custom data tracks to display in the UCSC Genome Browser.

Using the UCSC Genome Browser with KaryoStudio

You can use KaryoStudio to see what other genomic features sit within a found region. All analyses are based on chromosome and position. The UCSC Genome Browser shows you information about:

- RefSeq genes
- mRNAs
- ESTs
- genes in other organisms

There are also other options within the page that allow you to add or remove additional data tracks of information.

The UCSC Genome Browser may take awhile to load. Be patient while your data is loading; it will eventually display.

At the top, the chromosome and position are identified.

Next, information about RefSeq genes is listed.

Next, there is mRNA and EST information, which is useful for looking at locations of specific gene transcripts.

Next, the conservation of this gene in mammalian species is shown on a graph.

Next, the conservation of this gene in other species is shown on tracks.

Finally, the location of SNPs in humans, and the location of repeated DNA sequences.

Below all of this, you can select various additional information to include in this graph.

The Phenotype and Disease Association Studies track might be of interest. Select these tracks to see if any known phenotypes have been associated with this specific region.

PubMed

PubMed, available via the NCBI Entrez retrieval system, is hosted by the National Center for Biotechnology Information (NCBI) at the National Library of Medicine (NLM) at the U.S. National Institutes of Health (NIH). PubMed provides access to citations from biomedical literature and is a streamlined way to determine if there are any peer-reviewed and published studies related to the region, condition, or phenotype under study.

Using PubMed with KaryoStudio

Since this database is based on specific key words, not on chromosome number and position, KaryoStudio provides only a link to the homepage of this database.

If KaryoStudio has identified a specific phenotype within the found regions table, you can enter the keyword in the homepage of PubMed to obtain more information.

This section is similar to OMIM in that it is based on keywords. Type in a gene name, condition name, etc. This brings up a list of scientific publications in which the key word has been found.

Note that the more common your inquiry, the more hits you will get. To reduce the number of hits, make your search term as specific as possible.

DECIPHER

The DECIPHER consortium is a network of clinical genetic centers. KaryoStudio allows you to examine a specific found region of interest against this cytogenetics database of clinical cytogenetics cases by right-clicking on the found region and sending it to the database.

If a particular portion (or the whole region) of your found region has been previously linked to a certain phenotype, you can view that here. From the DECIPHER database, you can view your found region within the Ensembl Genome Browser, identify the type of array or technology used to identify the specific region, and obtain a DECIPHER Syndrome Report for a specific phenotype of interest, including a clinical description, references, affected genes, and a phenotype report.

Using DECIPHER with KaryoStudio

To use DECIPHER with KaryoStudio, do the following:

- Right-click a found region and select **DECIPHER** from the context menu (Figure 85).

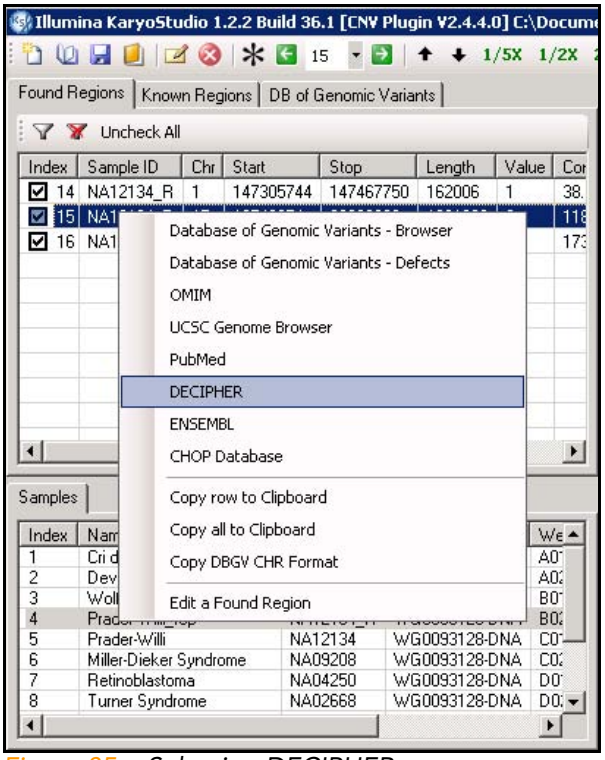


Figure 85 Selecting DECIPHER

KaryoStudio automatically sends information about the found region to DECIPER, and opens the DECIPHER window for that region (Figure 86).

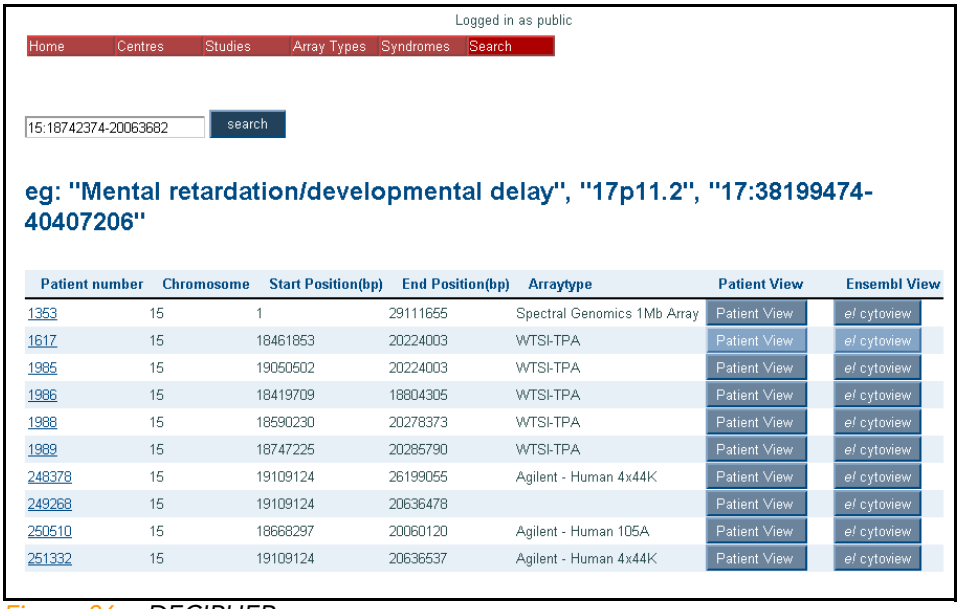

Logged in as public
Home
Centres
Studies
Array Types
Syndromes
Search
15:18742374-20063682
search
eg: "Mental retardation/developmental delay", "17p11.2", "17:38199474-40407206"
Patient number
Chromosome
Start Position(bp)
End Position(bp)
Arraytype
Patient View
Ensembl View
1353 15 1 29111655 Spectral Genomics 1Mb Array Patient View e! cytoview
1617 15 18461853 20224003 WTSI-TPA Patient View e! cytoview
1985 15 19050502 20224003 WTSI-TPA Patient View e! cytoview
1986 15 18419709 18804305 WTSI-TPA Patient View e! cytoview
1988 15 18590230 20278373 WTSI-TPA Patient View e! cytoview
1989 15 18747225 20285790 WTSI-TPA Patient View e! cytoview
248378 15 19109124 26199055 Agilent - Human 4x44K Patient View e! cytoview
249268 15 19109124 20636478 Patient View e! cytoview
250510 15 18668297 20060120 Agilent - Human 105A Patient View e! cytoview
251332 15 19109124 20636537 Agilent - Human 4x44K Patient View e! cytoview

Figure 86 DECIPHER

Ensembl

KaryoStudio allows you to examine your found region within the cytogenetics view of the Ensembl Genome Browser. Ensembl is a useful database for examining characterized disorders that may have been previously linked to your region.

Using Ensembl with KaryoStudio

To use Ensembl with Karyostudio, do the following:

- Right-click a found region and select **Ensembl** from the context menu (Figure 87).

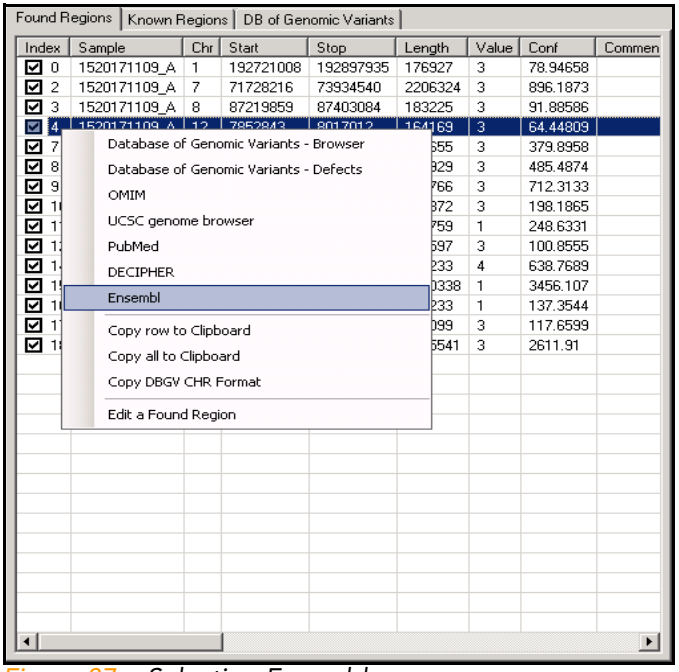


Figure 87 Selecting Ensembl

Karyostudio automatically sends information about your region to the Ensembl database, and the Ensembl Human CytoView window appears (Figure 88).

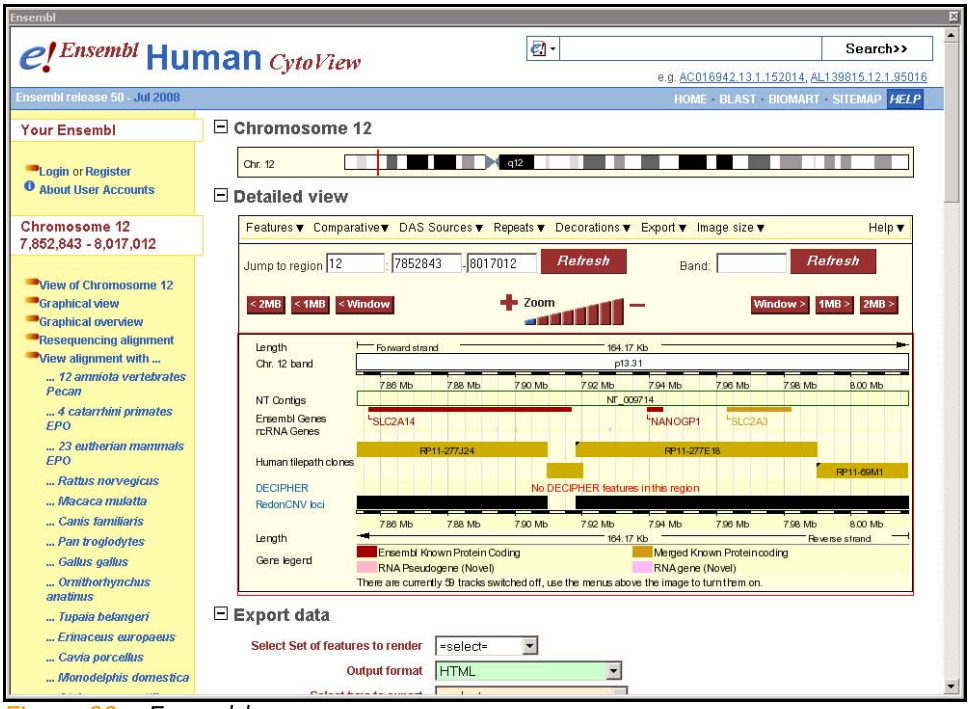


Figure 88 Ensembl

CHOP Database

The Copy Number Variation project at the Children's Hospital of Philadelphia (CHOP) represents an effort to identify all frequent copy number variations (CNVs) that exist in the human genome. The database currently consists of data from over 2,000 healthy individuals. This database was originally described in the following manuscript:

TH Shaikh et al. *(2009)* High-resolution mapping and analysis of copy number variations in the human genome: A data resource for clinical and research applications. *Genome Res 19: 1682-1690; doi:10.1101/gr.083501.108*

Using the CHOP CNV Database with KaryoStudio

To use the CHOP Database with KaryoStudio, do the following:

- Right-click a found region and select **CHOP Database** from the context menu (Figure 89).

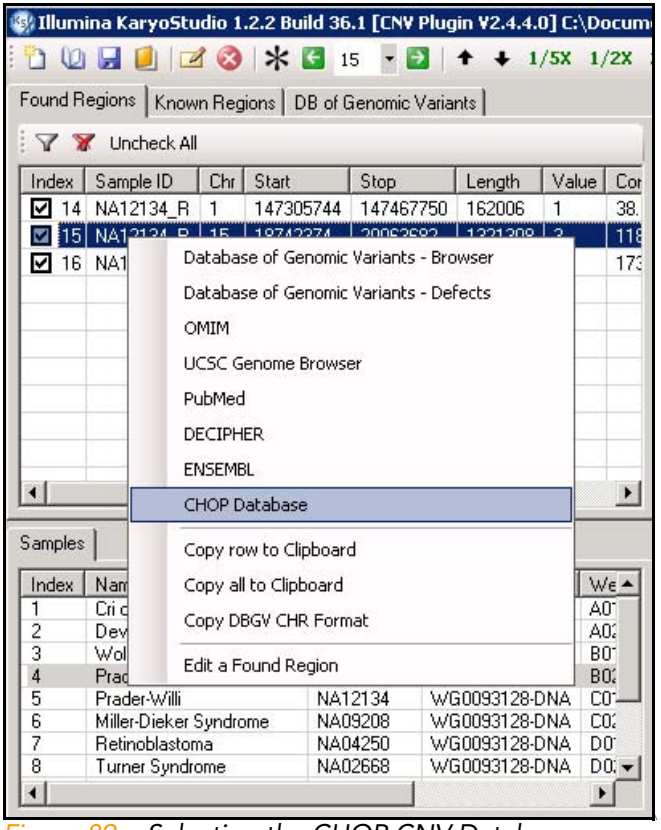


Figure 89 Selecting the CHOP CNV Database

KaryoStudio automatically sends information about the found region to the CHOP Database and opens the CHOP CNV browser window to display any CNVs in the CHOP Database that map to the region you submitted (Figure 90).

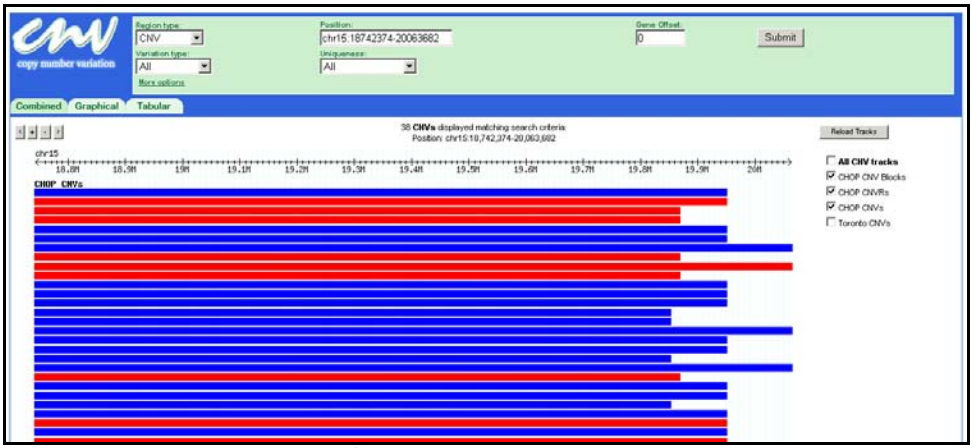


Figure 90 CHOP CNV Database CNVs

Chapter 6

Generating a Cytogenetics Report

Topics

112 Introduction

112 What's in a Cytogenetics Report?

116 Adjusting the Information in a Cytogenetics Report

116 Generating a New Cytogenetics Report

Introduction

KaryoStudio allows you to create a *.pdf Cytogenetics Report for each sample, summarizing the aberrations found in that sample and showing whether they cross-match with any known regions in the genome. Depending upon how many aberrations are found for a sample, and how many you choose to include in the report, it can be from one page to many pages long.

You need a recent version of Adobe Reader to view *.pdf reports. Adobe Reader is available free from get.adobe.com/reader/.

What's in a Cytogenetics Report?

A Cytogenetics Report contains all of the essential information required to quickly summarize aberrations found within a specific sample. Within each report is an entry for each found region, including an image of the aberration, its size, an estimate of the copy number, the confidence value (relative score), and a list of the disorders that have already been associated with that specific region.

Figure 91 shows an example Cytogenetics Report. Table 9 lists the elements of a report and identifies the elements that are adjustable.

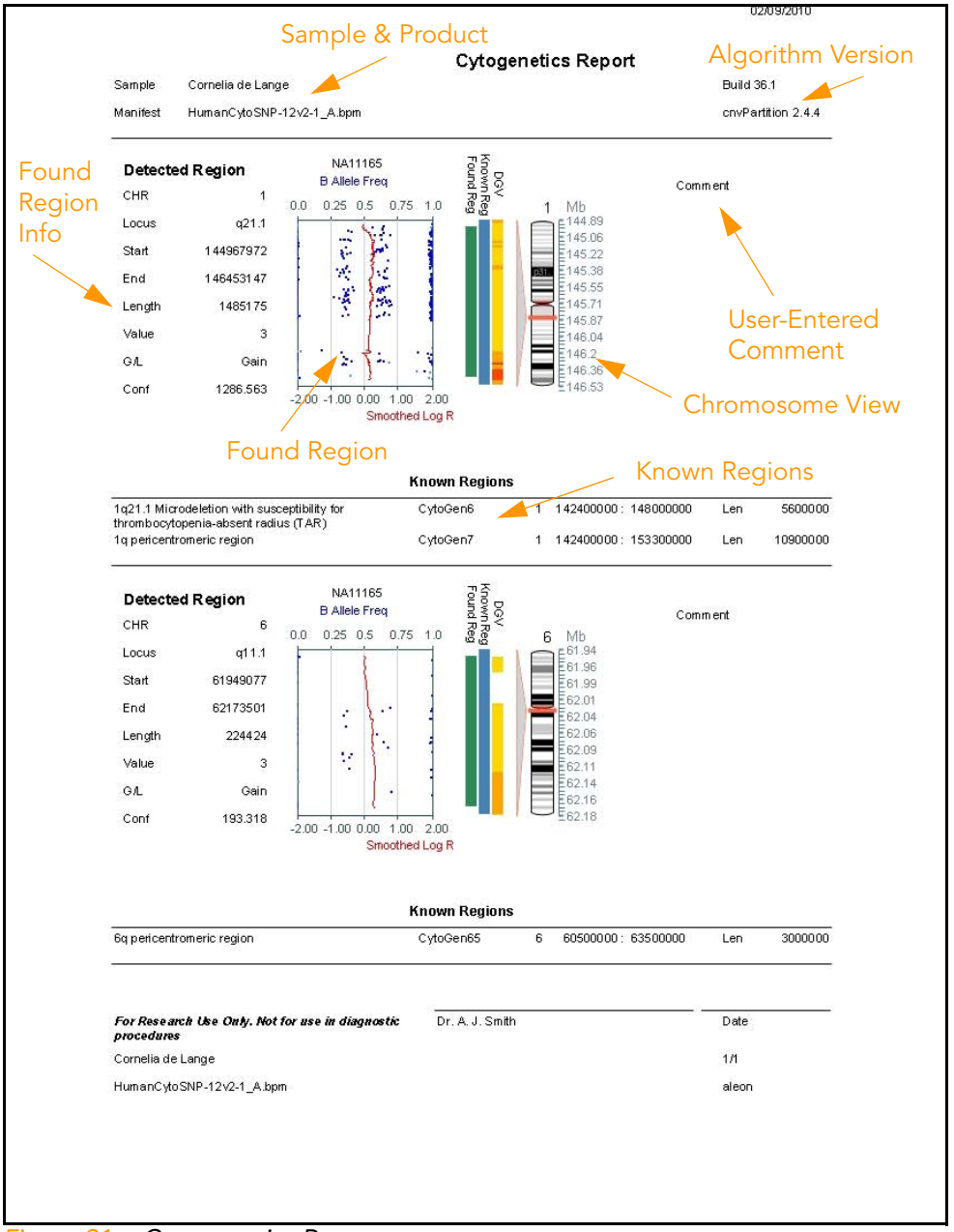


Figure 91 Cytogenetics Report

Table 9 Cytogenetics Report - Header

Column	Description
Date	Date the report was generated
Sample Name	Name of the sample presented in the report
Product Manifest	Product manifest used to create the project
Genome Build	Genome Build version used for analysis
cnvPartition Algorithm Version	Algorithm version used for analysis (not necessarily the most recently installed version)
Image of Found Region from Chromosome Browser	Yes. Can adjust number of images shown per report.

Table 10 Cytogenetics Report - Detected Region Information

Column	Description
CHR (Chromosome)	Chromosome on which the found region is located
Locus	Cytoband(s) on which the found region is located
Start	Start coordinate of the found region
End	End coordinate of the found region
Length	Length of the found region, measured in base pairs
Value	Copy number of the found region
G/L	Gain or loss
Conf	Confidence score for the found region, calculated by the algorithm

Table 10 Cytogenetics Report - Detected Region Information

Column	Description
Data plot	Plot of B Allele Freq and Log R of markers in the found region
Regions tracks	Found Region, Known Region, and DGV tracks from the chromosome browser
Coordinate ruler	Chromosomal coordinates of the data plot in megabases
Comment	User-defined comments for the found region. Can be left blank.
Known regions	Regions known to overlap with the found region. Includes Region Name, Region ID, Chromosome, Start coordinate, End coordinate, and Length.

Table 11 Cytogenetics Report - Footer

Column	Description
Sample Name	Name of the sample presented in the report
Manifest	Product manifest used to create the project
Signature and date	Area for the investigator to sign and date the report
Username	Name of the user logged into the system when the report was generated

Adjusting the Information in a Cytogenetics Report

You can adjust the information included in a Cytogenetics Report by using the KaryoStudio **Settings** tab.

You can adjust the number of aberrations included in a Cytogenetics Report by entering a value for *MaxReportedDefects*. On average, KaryoStudio includes roughly two aberrations per page, so a report with ten aberrations would be approximately five pages long.

You can limit the number of known regions to display for each found region by entering a value for *MaximumNumberOfKnownDefects*. If you do not want to cross-match to any known regions, set *MaximumNumberOfKnownDefects* to 0.

Additionally, you can select which found regions to include in a Cytogenetics Report by selecting the checkbox to the left of each found region in the Found Regions table. Clear the checkbox to the left of a found region to exclude it from a report.

Generating a New Cytogenetics Report

After you choose the parameters for the aberration filter and number of aberrations for each report, click **Write Reports to Directory**. A dialog box opens, allowing you to browse to and select a directory where you want the reports to be saved.

KaryoStudio generates the reports in the location you selected. This takes roughly 30 seconds to one minute per sample.

Appendix A

Additional Information

Topics

118 Introduction

118 System Information

119 cnvPartition Algorithm

120 B Allele Frequency and Log R Ratio

Introduction

This appendix includes information about additional topics that you may be interested in.

System Information

Use the following information as a guide to determine the minimum and recommended system configuration for the computer on which you plan to install KaryoStudio.

The minimum and recommended system configurations vary depending on the products you plan to use to generate the data you load into KaryoStudio.

Table 12 System Information

	Human CytoSNP-12	Human 610-Quad	Human 1M-Duo	HumanOmni1-Quad
Minimum System Requirements	32-bit	32-bit	64-bit	64-bit
Operating System	WinXP SP2 or Vista	WinXP SP2 or Vista	WinXP SP2 or Vista	WinXP SP2 or Vista
Minimum / Recommended Processor Requirements	Pentium IV 1.5 GhZ / Pentium IV 2.0 GhZ	Pentium IV 1.5 GhZ / Pentium IV 2.0 GhZ	Pentium IV 1.5 GhZ / Pentium IV 2.0 GhZ	Pentium IV 1.5 GhZ / Pentium IV 2.0 GhZ
.NET Version	.NET 3.5	.NET 3.5	.NET 3.5	.NET 3.5
Network Connection	1 GB	1 GB	1 GB	1 GB
Minimum Memory Requirements	4GB	4GB	8GB	8GB

Table 12 System Information (continued)

	Human CytoSNP-12	Human 610-Quad	Human 1M-Duo	HumanOmni1-Quad
Minimum Hard Drive Requirements	100GB	100GB	100GB	100GB
Minimum / Recommended Video Display Requirements	1024x768 / 1280x1024	1024x768 / 1280x1024	1024x768 / 1280x1024	1024x768 / 1280x1024

cnvPartition Algorithm

The cnvPartition algorithm automatically scans your data when you create a new project. cnvPartition v2.4.4, provided with KaryoStudio v1.2, has been designed to scan for deletions, duplications, and amplifications on the autosomes and sex chromosomes.

The cnvPartition algorithm is based on a recursive partition method. cnvPartition, which has been optimized for speed and accuracy, uses log R intensity and B Allele Freq for identification of chromosomal aberrations, estimates copy number values, and calculates per locus confidence scores. After you load your data into KaryoStudio, cnvPartition will automatically scan the data for aberrations.

For more information about how this algorithm works, please see the DNA Copy Number Analysis Algorithms Technical Note at http://www.illumina.com/Documents/products/technotes/technote_cnv_algorithms.pdf.

Confidence Score

The confidence score that cnvPartition generates is defined as the sum of all logged likelihoods in the region for the assigned copy number minus the sum of all logged likelihoods of copy number equal to 2 for loci in the region. Thus the confidence scores provide a means to rank regions relative to their (dis)similarity to normal, copy number 2 segments. Higher values represent higher confidence in the aberration call.

For more information about recommended confidence score cutoff values, see the KaryoStudio FAQs on http://www.illumina.com.

B Allele Frequency and Log R Ratio

B Allele Frequency and Log R Ratio data form the basis of the analysis of all copy number changes in Infinium data. B Allele Frequency data is derived from SNP genotypes, while Log R Ratio data is derived from intensity information.

Calculation

The B Allele Freq for a sample shows the theta value for a SNP, corrected for cluster position. Cluster positions are generated from a large set of normal individuals. The B Allele Frequency can also be referred to as "copy angle" or "allelic composition." It is easier to visualize genotyping data for all SNPs within a chromosomal region using B Allele Freq rather than theta values. This is true because B Allele Freq exhibits less locus-to-locus variation than the theta values for a given sample. The transformation of theta values to allele frequencies allows for improved measurements and better visualization of both LOH and copy number changes.

B allele freq is described by the following equations.

B allele freq

= 0 if theta < tAA

= 0.5 * (theta - tAA) / (tAB - tAA) if theta < tAB

= 0.5 + 0.5 * (theta - tAB) / (tBB - tAB) if theta < tBB

= 1 if theta >= tBB

where:

- tAA = mean theta value of all genotypes in the AA cluster plotted in polar normalized coordinates
- tAB = mean theta value of all genotypes in the AB cluster plotted in polar normalized coordinates
- tBB = mean theta value of all genotypes in the BB cluster plotted in polar normalized coordinates

The Log R Ratio is based on normalized intensity data and for a sample is the log (base 2) ratio of the normalized R value for the marker divided by the expected normalized R value. For loci included in GenomeStudio statistics such as Call Rate, the expected R value is computed by linear interpolation of the R value at the SNP's theta value for a sample, relative to the R values of the surrounding clusters. Because no clusters are generated for loci in the "Intensity Only" category, the Log R Ratio for these loci is adjusted so that the expected R value is based on the weighted mean of the cluster itself. Log R Ratio is displayed the same way for these loci as it is for loci included in GenomeStudio statistics in tools such as the IGV. Both SNPs and intensity-only loci such as nonpolymorphic probes (which usually have the identifier "cnv" in their names) are displayed in the log R ratio plots in KaryoStudio.

For example, if for a given sample and SNP with:

- A theta value of 0.2
- an AA cluster at theta = 0.1, R = 1.5
- an AB cluster at theta = 0.4, R = 2.5

The estimated R at theta for the sample is: 0.2 is 1.5 + (0.2-0.1) * (2.5-1.5) / (0.4-0.1) = 1.83. If the R value for the SNP is 1.6, the Log R Ratio is: log2 (1.6/1.83) = -0.196

Interpretation

cnvPartition automatically scans both the B allele frequency and log R ratio data for the presence of aberrations. In regions of the genome with two copies, the B allele frequency sits at 0, 0.5, and 1 representing the AA, AB, and BB genotype clusters. In regions of the genome that do not have two copies, various patterns may be seen. You must take into account the direction in which the log R ratio is deflected to determine if a gain or loss of DNA is present. Increases in the log R ratio indicate duplications (or amplifications) and decreases indicate deletions.

For more information about interpreting this data, see the following Technical Note: Interpreting Infinium Assay Data for Whole-Genome Structural Variation.

Appendix B

Sample Sheet

Topics

124 Introduction
124 Sample Sheet

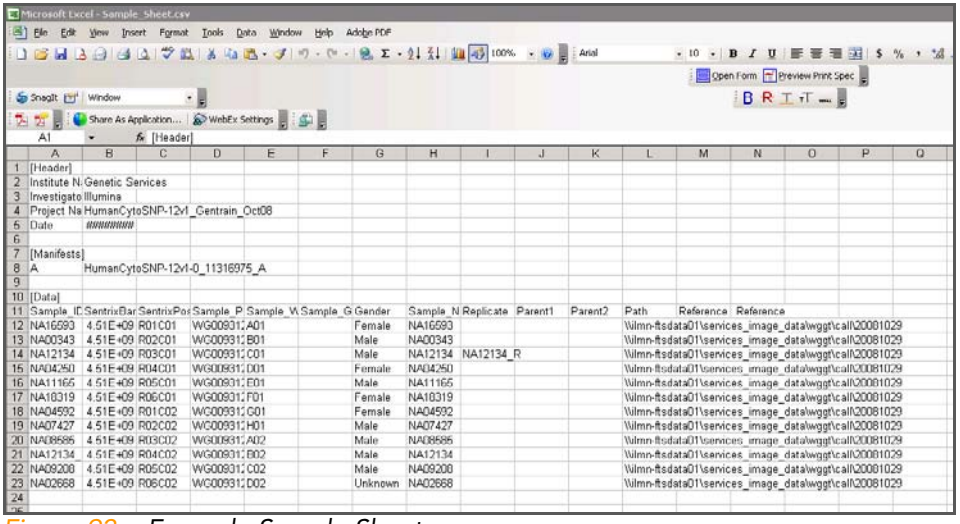
Introduction

The figure below is an example Sample Sheet for KaryoStudio. The following columns are required:

- Sample_ID
- SentrixBarcode_A
- SentrixPosition_A
- gender

All other columns are optional. If path is not provided in the sample sheet, it will be required in the new project wizard.

Sample Sheet



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	[Header]																
2	Institute N	Genetic Services															
3	Investigato	Illumina															
4	Project Na	HumanCytoSNP-12v1_Centrain_Oct08															
5	Date	##########															
6																	
7	[Manifests]																
8	A	HumanCytoSNP-12v1-0_11316975_A															
9																	
10	[Data]																
11	Sample_ID	SentrixBar	SentrixPos	Sample_P	Sample_W	Sample_G	Gender	Sample_N	Replicate	Parent1	Parent2	Path	Reference	Reference			
12	NA16593	4.51E+09	R01C01	WG00931	A01		Female	NA16593				\\ilmn-ftsdata01\services_image_data\wggt\call\20081029					
13	NA00343	4.51E+09	R02C01	WG00931	B01		Male	NA00343				\\ilmn-ftsdata01\services_image_data\wggt\call\20081029					
14	NA12134	4.51E+09	R03C01	WG00931	C01		Male	NA12134	NA12134_R			\\ilmn-ftsdata01\services_image_data\wggt\call\20081029					
15	NA04250	4.51E+09	R04C01	WG00931	D01		Female	NA04250				\\ilmn-ftsdata01\services_image_data\wggt\call\20081029					
16	NA11165	4.51E+09	R05C01	WG00931	E01		Male	NA11165				\\ilmn-ftsdata01\services_image_data\wggt\call\20081029					
17	NA10319	4.51E+09	R06C01	WG00931	F01		Female	NA10319				\\ilmn-ftsdata01\services_image_data\wggt\call\20081029					
18	NA04592	4.51E+09	R01C02	WG00931	G01		Female	NA04592				\\ilmn-ftsdata01\services_image_data\wggt\call\20081029					
19	NA07427	4.51E+09	R02C02	WG00931	H01		Male	NA07427				\\ilmn-ftsdata01\services_image_data\wggt\call\20081029					
20	NA08585	4.51E+09	R03C02	WG00931	A02		Male	NA08585				\\ilmn-ftsdata01\services_image_data\wggt\call\20081029					
21	NA12134	4.51E+09	R04C02	WG00931	B02		Male	NA12134				\\ilmn-ftsdata01\services_image_data\wggt\call\20081029					
22	NA09200	4.51E+09	R05C02	WG00931	C02		Male	NA09200				\\ilmn-ftsdata01\services_image_data\wggt\call\20081029					
23	NA02668	4.51E+09	R06C02	WG00931	D02		Unknown	NA02668				\\ilmn-ftsdata01\services_image_data\wggt\call\20081029					
24																	

Figure 92 Example Sample Sheet

Appendix C

Known Regions File

Topics

126 Introduction

126 Known Regions File

Introduction

Table 13 lists and describes the required columns of a Known Regions File for use with KaryoStudio.

Table 13 Known Regions File Column Descriptions

Column	Description
Region#	ID code for the region
Disorder	Text description of the significance of the region
Chr	Chromosome number of the region
Start	Start coordinate of the region
End	End coordinate of the region
Length	Length of the region in base pairs
Length minus overlap	Harper to provide info

The figure below is an example Known Regions File.

Known Regions File

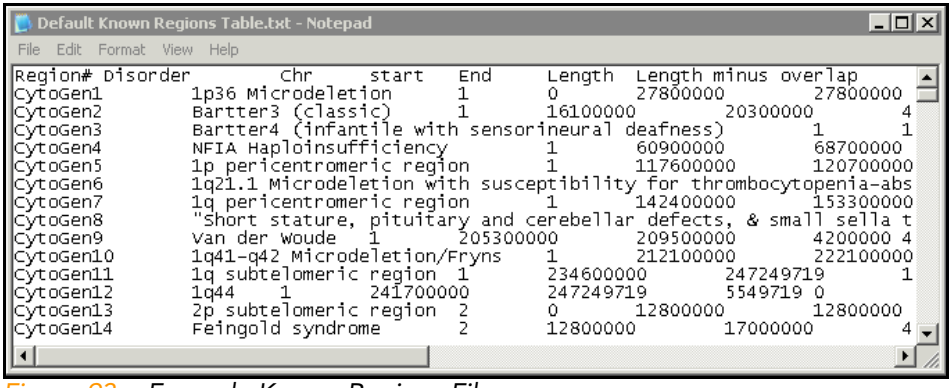

Default Known Regions Table.txt - Notepad
File Edit Format View Help
Region# Disorder Chr start End Length Length minus overlap
CytoGen1 1p36 Microdeletion 1 0 27800000 27800000
CytoGen2 Bartter3 (classic) 1 16100000 20300000 4
CytoGen3 Bartter4 (infantile with sensorineural deafness) 1 1
CytoGen4 NFIA Haploinsufficiency 1 60900000 68700000
CytoGen5 1p pericentromeric region 1 117600000 120700000
CytoGen6 1q21.1 Microdeletion with susceptibility for thrombocytopenia-abs
CytoGen7 1q pericentromeric region 1 142400000 153300000
CytoGen8 "Short stature, pituitary and cerebellar defects, & small sella t
CytoGen9 Van der Woude 1 205300000 209500000 4200000 4
CytoGen10 1q41-q42 Microdeletion/Fryns 1 212100000 222100000
CytoGen11 1q subtelomeric region 1 234600000 247249719 1
CytoGen12 1q44 1 241700000 247249719 5549719 0
CytoGen13 2p subtelomeric region 2 0 12800000 12800000
CytoGen14 Feingold syndrome 2 12800000 17000000 4

Figure 93 Example Known Regions File

Appendix D

Ignored Regions File

Topics

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128 Ignored Regions File

Introduction

Table 14 lists and describes the required columns of an Ignored Regions File for use with KaryoStudio.

Table 14 Ignored Regions File Column Descriptions

Column	Description
Region Name	Text description of the region
Chr	Chromosome number of the region
Start	Start coordinate of the region
Stop	Start coordinate of the region

The figure below is an example Ignored Regions File.

Ignored Regions File

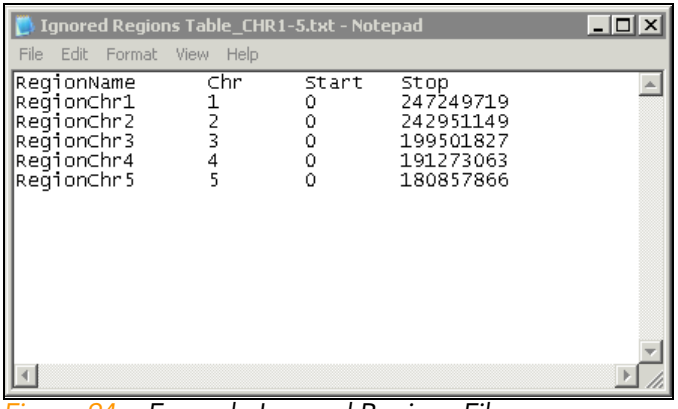
Ignored Regions Table_CHR1-5.txt - Notepad
File Edit Format View Help
RegionName Chr Start Stop
RegionChr1 1 0 247249719
RegionChr2 2 0 242951149
RegionChr3 3 0 199501827
RegionChr4 4 0 191273063
RegionChr5 5 0 180857866

Figure 94 Example Ignored Regions File